AFFLICTION WITH TOXOPLASMOSIS AMONG INFERTILE MEN IN KAMAL AL-SAMARAEE HOSPITAL, BAGHDAD CITY [+]

Ahmed A. M. Al-Saadi [□] **Mohammed J. Qasim** [□□] **Nuhal Noel Jebri** [□□□]

Abstract :

Toxoplasmosis is a zoonotic disease of human and animal, caused by the opportunistic protozoan *Toxoplasma gondii.* The present study was performed in Kamal AL-Samaraee Hospital, in Baghdad city during the period from the first of October 2011 till the end of January 2012. In this cross sectional study, ELISA technique was used for the detection of IgM and IgG in 110 infertile men aged from 25 to 59 years. Out of the total number of infertile men, the results showed that 46 (41.81%) were positive for toxoplasmosis, with a higher frequency in the age group (35-39) years. Among those seropositive results, there were 43cases (93.47%) with decreased level of testosterone, 26 cases (56.52%) with abnormal sperms morphology, 31cases (67.39%) with decreased sperm count and 34 cases (73.91%) with abnormal sperm motility.

الإبتلاء بداء المقوسات لدى الذكور عديمي الخصوبة في مستشفى كمال السامرائي , مدينة بغداد

احمد عبد الامير محمود السعدي محمد جاسم قاسم نهال نوئيل جبري

المستخلص:

داء المقوسات هو أحد الامراض المشتركة بين الانسان والحيوان ، والذي تسببه مقوسة كوندای الاحادية الخلية الانتهازية المعيشة . أُجريتْ الدراسة الحالية في مستشفى كمال السامرائي في بغداد للمدة من بداية تشرين الاول 2011 وحتى نهاية كانون الثاني 2012 . في هذه الدراسة المقطعية , تم استعمال مقايسة المميز المناعي المرتبط بالانزيم (ELISA) لتحديد الكلوبيولين المناعي (IgM) و (IgG) لمائة وعشرة من الرجال الذين يعانون عدم الخصوبة وقد تراوحتْ اعمارهم ما بين 19–59 سنة . أظهرت النتائج أن (41.81%) 46 منهم كانوا ايجابيين للفحص المصلي لداء المقوسات مع أعلى نسبة تكرار مابين الفئة العمرية (35–39) سنة.من بين الحالات الموجبة لفحص داء المقوسات المصلي كان هناك 43 حالة (93.47%) بمستوى متدني لهرمون الذكورة , و 26 حالة (56.52%) بإشكال غير طبيعية للنطف , و 31 حالة(67.39%) بإعداد متدنية للنطف, و34 حالة (73.91%) بحركة غير طبيعية للنطف.

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[*]Prof / College of Health and Medical Technologies

[**]Assist. Lecturer /College of Health and Medical Technologies

[***]Kamal Al-Samaraee Hospital

Introduction:

Toxoplasma gondii is an intracellular protozoan parasite that infects humans and animals. Infection by *T. gondii* is widely prevalent in man and animals throughout the world, and of both veterinary and medical importance, because it may cause abortion or congenital disease in its intermediate hosts [1] . The main source of the infection for humans is from the consummation of raw or undercooked meat of intermediate host (especially pigs, sheep and rabbits) containing tissue cysts and from food or water contaminated with cat feces containing sporulated oocysts [2].

Toxoplasmosis is one of the classical conditions known to have a profound adverse effect on human reproductive functions [3]. Furthermore, it has been recently reported that children with congenital toxoplasmosis have a high frequency of precocious puberty [4]. Acute *Toxoplasma* infection may cause temporary hypogonadotrophic gonadal insufficiency regardless of the course of the disease [5]. Several conditions can interfere with spermatogenesis and reduce sperm quality and production. More factors such as drug treatment, chemotherapy, toxins, infections, air pollutions and insufficient vitamins intake, parasites such as *T. gondii* tachyzoites have harmful effects on spermatogenesis and sperm normal production [6].

Toxoplasma infection changes the concentration of serum testosterone in mice and human rather than changed concentration of testosterone influences the probability of the *Toxoplasma* infection, it is possible that the decrease of testosterone is an adaptive mechanism of infected mice aimed to compensate toxoplasmosis-induced immunosuppression observed during latent *Toxoplasma* infection [7].

Toxoplasmosis can affect main reproductive parameters in male rats including sperm motility, concentration and morphology, which are the most predictive of their fertilizing capacity. White rats, which are considered to be the best model for human's toxoplasmosis due to their natural resistance to *Toxoplasma* infection, may be used for further investigation on the possible effect of toxoplasmosis on man's fertility[8]. This study is aimed to detect the affliction with Toxoplasmosis among infertile men, determine the disturbance in Testosterone levels and observe the sperm's motility, morphology, and count among infected men.

Materials and methods:

This cross sectional study was done on 110 infertile males which visited Kamal Al-Samaraee Hospital, the center of fertility and in vitro fertilization in Baghdad city, during the period from the first of October 2011 till the end of January 2012.

1- Blood Collection:-

Five milliliters (5mL) of blood were collected by vein puncture using syringe with needle gauge 23, transported to unhepranized tube and allowed to clot at room temperature and sera were separated by centrifugation at 1500g for 5 min, then stored and frozen at (– 20^{o}C) [9].

2- Serological Tests:-

Enzyme linked immunosorbent assay test was run for all the samples using lgM and lgG Kits (BioCheck, Inc., USA) and final results were recorded by ELISA reader (optical absorbance,

OD=450). Samples less than (0.90) unit/ml, (0.91-.099) unit/ml and (1.00) unit/ml or greater were considered as negative, suspicious and positive, respectively.
Toxoplasma seropositive infertile men were examined by using VIDAS (BioMerieux kit, France) method for the measurement of testosterone levels in their sera. According to this kit, the normal level of testosterone in men serum should be 3-10.6 ng/ml, so patients less or more than this level were considered as abnormal.

3- Semen Collection:-

The semen samples were collected after a minimum of 3 days of sexual abstinence, and then every patient was given a clean, wide mouth, sterile, dry, graduated plastic and warm disposable container. The container was labeled with the man's name, identification number, and the date and time of collection. After masturbation the seminal fluid samples were immediately incubated at 37°C, and waiting for complete liquefaction (during 30 minutes) [10].

4- Semen Analysis:-

After liquefaction of the sample, sperms motility, morphology and count of *Toxoplasma* serospositive infertile men were assessed according to WHO standard procedures [10].

4.1 Sperms Motility:-

A standard volume of semen,(10 μl) was placed onto a clean glass slide which was examined with phase-contrast optics at ×200 or × 400 magnifications. Men with progressive motility (PR) less than 32% were suffered from abnormality in their sperms motility.

4.2 Sperms Count:-

The count of spermatozoa was determined by using the improved Neubauer haemocytometer. The haemocytometer was examined with phase-contrast optics at ×200 or × 400 magnifications. One chamber grid by grid, was examined and continued counting until at least 200 spermatozoa had been observed. The lower reference limit for sperms concentration is 15×10^6 spermatozoa per ml.

4.3 Sperms Morphology:-

Preparing a smear of semen on a slide, then air-drying, fixing and staining with Papanicolaou stain the slide were done. The slide was examined with bright field optics at ×1000 magnification. The lower reference limit for normal forms is 4%. Sperms with abnormal shape more than 4% were considered abnormal.

5- Statistical Analysis

Statistical comparisons were made using the binomial test for comparison of data in the positive and negative males, normal to abnormal parameters of their sperms and their abnormality of testosterone levels to abnormality in sperms. Descriptive analysis was used in order to analyze and assess the mean value and standard deviation of age groups. All of the statistical analyses were performed by SPSS version 10.0 [11]

Results:

Out of 110 infertile men, the *toxoplasma* seropositive were 46 (41.81%) and negative cases were 64 (58.18%). The comparison of positive and negative men frequency was non significant which indicating that these two groups were similar in occurrence (Table 1).

Table 1:Frequency of positive and negative infertile men as measured by *Toxoplasma* ELISA.

Infertile Men	Toxoplasmosis		Total (No.) & (%)	P-value (Binomial Test)
	Positive	Negative		
No.	46	64	110	P=0.105 (NS)
%	41.81	58.18	100	

Table (2) shows, the distribution of Toxoplasma seropsitive cases according to their ages. The highest frequency of seropositive cases was noticed among the age group 35-39 years (14/46) represented (30.43%) of the total with a mean age value of 40.00±7.94SD.

Table 2: Distribution of the Toxoplasma seropositive Infertile Men According to the Age.

Age Groups in the Positive Men (years)	No.	%	Mean & Std. Deviation
25 – 29	2	4.34	40.00±7.94
30 – 34	8	17.39	
35 – 39	14	30.43	
40 – 44	9	19.56	
45 – 49	6	13.04	
50 ≥	7	15.21	
Total	46	100	

According to table 3, there were 43 (93.47%) of *Toxoplasma* seropsitive infertile men with decreasing testosterone levels, while only one (2.17%) was with increased levels, two cases(4.34%) were with normal testosterone level.

Table 3: Demonstration of the Testosterone Levels in the *Toxoplasma* seropsitive Infertile Men.

Testosterone Hormone in Positive Men	Levels	No.	%
Normal levels (3-10.6 ng/ml)	Normal	2	4.34
	Abnormal (decreased)	43	93.47
	Abnormal (increased)	1	2.17
Total		46	100

Out of total number (46) of *Toxoplasma* seropsitive infertile men , table 4 shows that 26 (56.52%) were with abnormal sperm morphology and 20 (43.47%) were with normal in sperm morphology. Sperms count (concentration per ml) in 31 (67.39%) of infertile men *Toxoplasma* seropositive was abnormal versus 15 (32.60%) men were normal. Abnormal sperm motility was

observed in 34 men with percent (73.91) whereas 12 men were with normal sperm motility with percent (26.08%).

The comparison significance (C.S.) between normal and abnormal men in sperm morphology was non significant (p-value= 0.461), and between men with normal and others with abnormal sperm concentration was significant (p-value= 0.026), while it was highly significant in case of sperm motility between normal and abnormal men in this semen parameter.

Table 4: Demonstration of the Normal and Abnormal Semen Analysis in the Positive Infertile Men.

Semen analysis in the positive men	No. & %	Abnormal	Normal	C.S. P-value (Binomial Test)
Sperm Morphology	No.	26	20	P= 0.461 (NS)
	%	56.52	43.47	
Sperm count (Concentration)	No.	31	15	P= 0.026 (S)
	%	67.39	32.60	
Sperm Motility	No.	34	12	P= 0.002 (HS)
	%	73.91	26.08	

Discussion:

Up to our knowledge, this is the first study- at least in Iraq - which is trying to correlate between affliction with toxoplasmosis and infertility in men and to investigate about the disturbance in testosterone hormone levels and sperms disorder (motility, morphology and count) in positive infertile men.

In the current study, we reported the observations that increased our understanding of the abrupt effect of *T. gondii* infection on human reproductive function. These observations were in line with previous results of experimental studies in animals and limited studied in human. According to several studies, *T. gondii* has been isolated from caprine [12], ovine[13], bovine [14], swine [15], and human semen [16,17].

The present study demonstrated that the presence of toxoplasmosis in infertile males is not rare. There were 46/110 (41.81%) of them had afflicted with toxoplasmosis. This result agreed with other study in which the prevalence of *Toxoplasma* infection among 100 cases of man's sterility in china , 36% of them were serologically *Toxoplasma*-IgG and IgM positive. These were concluded that *T. gondii* infection may affect men's fertility and cause sterility [18].

The result also agreed with study of [17] in Iraq, in which the rate of infection in men by using latex test, ELISA (IgG) and ELISA (IgM) was 82%, 66% and 28%, respectively.

This study found that the higher frequency of *Toxoplasm* seropositive men was in age group 35-39 years, and this result agreed with one study [19], in which 80% prevalence was recorded in 30-45 years the results of age group of Ontario Veterinary Medical Staff, Canada. Our result also was in agreement with another study [20] which demonstrated that the higher rate of *Toxoplasma* infection occurs in age group (30-39) years, while the results of another study [17] showed that the higher rate of infection was among infected men lie in the age group (25-31)years. This difference in the age groups of patients that afflicted with toxoplasmosis can be explained as the total number of samples in these studies were not equal and so as the factors that

interfere with the epidemiology of *T. gondii* such as feeding habits and cultural characteristics [21].

Out of 46 *Toxoplasma* seropositive males, 43 (93.47%) of them were with low levels of testosterone (table 3), This result was in agreement with other results in Czech Republic[7],as they showed that *Toxoplasma*-infected mice, both females and males, had lower serum testosterone concentration than controls. This can be explained as that the decrease of testosterone concentration could be an adaptive response of infected mice to *Toxoplasma*-induced immunosuppression, by this decreasing in the concentration of testosterone, the infected mice could partly compensate the latent toxoplasmosis-associated down-regulated cellular immunity. These results were similar to the results of an Egyptian study[22] as they showed that testosterone level in the serum of infected rabbits was highly decreased in comparison with that of the controlled group from the first week till the end of experiment.

This study disagreed with one study [23];which showed that a direct relation between the raise of *Toxoplasma* infection and cortisol is exist and testosterone would increases in both men and women. Men patient's results in this study were agreed with [5], which showed that 9 of 40 patients (22.5%) had lower levels of sex hormones (FSH, LH, Free Testosterone and Total Testosterone) than normal.

According to table 4, this study found that out of 46 *Toxoplasma* seropositive infertile men, men with abnormal morphology of sperms were 26 (56.52%), men with abnormal sperm count were 31 (67.39%) and those with abnormal sperm motility were 34 (73.91%). These results nearly agreed with another study [8], which conducted on Wistar males rats (*Rattus norvegicus*) in which the motility and concentration of sperm were significantly decreased in the infected group compared to controls, and a remarkable elevation up to 30% of spermatozoa abnormalities (bent tail, lost of hook shape, head lost, double head and cytoplasmic droplet) was noticed in infected group.

Another study [18] was in agreement with the current study resultes, in which it was found pathological changes in the testes, epididymis, vas deferens, prostate and thalamus of male mice with experimentally-induced acute *T. gondii* infection and they concluded that acute infection can cause infertility.

In this study it was observed that the frequency of abnormality of sperm (motility, count, and morphology) was more than normality. These results indicate that *T. gondii* may cause direct or indirect defect in sperm quality which is the main cause of infertility in men.

According to the present study, we conclude that *T. gondii* may be considered one of the different reasons which influence the levels of testosterone (decreasing) and causing sperms disorder (motility, morphology and count) in the infected men. So, our recommendation is that the IVF and fertility centers should be investigate about *T. gondii* infection in the serum and semen of infertile men as a routine test and treat the positive cases.

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