



## The considerable evidence between latent toxoplasma infection with testosterone and total antioxidant among infertile women

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### Abstract

*Toxoplasma gondii* an intracellular parasite that cause toxoplasmosis. Toxoplasma infection it's well known to induce various physiological, hormonal and behavioral changes in humans and animals. In current study, 147 infertile women were divided into three groups, primary, secondary infertility and aborted woman (sub-infertility). Twenty healthy volunteer women served as controls. Enzyme linked immunofluorescence assay was used to confirm anti-Toxoplasma IgG antibodies. Whereas, Testosterone concentration were measured by ELISA technique. We investigated the testosterone and total antioxidant capacity (TAC) in free and infected (*Toxoplasma gondii*) infertile women, to provide clinical scenario that may be adopted as one causes of primary, secondary infertility and sub infertility. Although, there are no significant variation in Testosterone levels between infertility women groups, but according normal, hypo and hyper range, testosterone were increased significantly just in toxoplasmosis women(p-value=0.04), whereas no significant variation are seen in other infertile groups. Testosterone statistically decreased in primary infertile women. In additions, serological analysis confirm that 28 (19%) enrolled women were seropositive for *Toxoplasma gondii*. Toxoplasmosis have a significant impact on Total antioxidant capacity especially in secondary infertility and sub-infertility. In addition no significant variations were reached in TAC and Testosterone as correlated with intensity of parasites (anti-IgG antibodies). interestingly the testosterone hyper concentration are found in secondary infertile women(p-value=0.01, 29%) and in opposite direction percentage of hypo-testosterone is increased significantly in primary infertility women. In conclusion this study suggested a considerable evidence of hypothesis that consider Toxoplasmosis is a one of a risk factor for increase the level of Testosterone and increasing the chance of infertility in women especially according Hypo-Hyper values. Also based our data that supplementations of TAC is recommended for women who had primary and secondary infertility to enhance reproductive outcome.

**Keywords:** *toxoplasma gondii*, testosterone, total antioxidant capacity, infertile women

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### INTRODUCTION

*Toxoplasma gondii* consider is one of the well-studied parasites because importance in medical and veterinary field, and its suitability as a model for molecular and cell biology studies (Dubey and Jitender 2008). Millions of parasite (oocysts) can shed into the environment by feline's definitive host. Depending on climatic conditions, the oocysts can survive for up to 18 months and are a source of infection for humans and animals. Another of transmission route is through ingested tissue cysts in uncooked or cured meat from animals that infected with *T. gondii* (Innes 2010, El-Shahaby et al. 2019). Although, asymptomatic most infected women, but congenital, neonatal death and physiological symptoms can present with acute

infections especially in first trimester (Sugden *et al.* 2016, Bamba, *et al.* 2017). Previous data showed the chronic *Toxoplasma gondii* infection induce various hormonal changes and manipulate behavioral in infected humans and animals and may be involved in etiology of different neurologic and psychiatric disorders (Zouei *et al.* 2018). Testosterone is much greater in men than women and it's involved in many reproductive physiology outcome in both males and females. Furthermore have morphology development, psychology and behavior roles (Brid and Briane

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2018). The researcher focussed on increase IgG anti-Toxoplasma antibodies and they correlated to testosterone. Most of these results revealed a different data according to *T. gondii* strain (Galván-Ramírez *et al.* 2014). Data in Previous showed *Toxoplasma gondii* can increase the number of son, as well as changes in men and women personality factors. Testosterone plays different roles in behavioral parameter the administration of exogenous testosterone can reduce fear in both humans and rodents (Abdoli and Amir 2014). Therefore, a testosterone changes can play an important roles in relation with *Toxoplasma* as aforementioned phenomena (Shahnaz *et al.* 2011). Mahbodfar *et al.* (2015) showed testosterone concentration increased significantly in relation to latent toxoplasmosis. Findings of different studies have a great deal of direct and indirect evidence of testosterone levels decrease in rodents and humans during acute toxoplasmosis (Abdoli and Amir 2014). The effect of latent toxoplasmosis on Testosterone productions is still controversial. Many published data have shown increased and decreased in Testosterone levels with latent toxoplasmosis in male and female human and experimental animals (abdoli 2014, Nirmala and Muruganandam 2019).

It is well known that Oxidative stress is the result of an imbalance between reactive oxygen species (ROS) productions and antioxidant defenses, the ROS are unstable and very reactive by-products of normal metabolism, causing damaging effects on the principal biomolecules (Fletcher *et al.* 2013, Schieber and Chandel 2012). Also, it's thought that Oxidative to be involved in the development of various diseases such as atherosclerosis, cancer, Alzheimer's, disease, diabetes, heart failure, autism and infection. Therefore, small changes in Oxidative and antioxidant can effect of the imbalance between oxidants and antioxidants that results in an abnormal redox state of cells (Tan *et al.* 2018). The principle host defense against intracellular parasite such as *Toxoplasma gondii* is activation of Macrophages by lymphokines were the rapid reactive oxygen species are release. (Bahrami *et al.* 2016, Razumovskaya *et al.* 2019) have done a research on *Toxoplasma gondii* infection on ROS and Total antioxidant capacity and their effect on enter the infection in chronic stage, they found both total antioxidant (decrease) and ROS increase, they conclude the antioxidant system one of effective mechanisms in tachyzoite-bradyzoite interconversion. Pervious controversial data have shown increase and decrease in Testosterone level with toxoplasmosis. As a former hypothesis the Testosterone immunosuppressive hormone, that lead to minimize the macrophage activation, finally this may be affected on Redox-antioxidant balance. The current aimed to investigate Testosterone level and total antioxidant capacity in latent toxoplasmosis infertile women to give

us understanding roles of antioxidant and testosterone and infertile developing in women.

## MATERIAL AND METHODS

### Subjects

#### Control (Healthy women)

All exclusion criteria, such as family history, chronic and genetic disease, drinking and smoking, have previous abortions were account to exclude women from control one. After an interviewer managed we used twenty enrolled women, ten as healthy non-pregnant and ten as healthy pregnant women.

#### Patients (infertile women)

One hundred seventy- four enrolled women had been complete clinical data and biochemical investigation for subdivided into four groups, primary, secondary and sub fertility as well as infected group.

#### Blood Collection

Five ML of blood collected from subjects and then blood transferred to gel tube. Serum was separated from whole blood by centrifugation (4000 rpm for 5 Min.) and then blood stored in refrigerator immediately at -20 C.

#### Seroprevalence of Toxoplasmosis

The detection of serum anti-toxoplasma IgG antibodies were identified by enzyme linked immunofluorescence assay (ELIFA) technique briefly, this assay principle combines an enzyme immunoassay method by immunocapture with final fluorescent detection (ELFA). The procedure of this assay accomplished by manufacture commercial kit by Biomerieux Company (France).

#### Testosterone Assessment

Testosterone concentration measured by using Enzyme-Linked Immunosorbent Assay (ELISA) technique by using commercial ELISA kit (Monobind Inc., USA).

#### Measurement of Total Antioxidant

Total serum antioxidant capacity was measured according to cupric method (Apake *et al.* 2004) briefly, the sample and standard act to reduce  $\text{Cu}^{++}$  to  $\text{Cu}^{+}$  is combined action of antioxidant. This reduced from copper will selectively appearance as a complex with the chromogenic reagent marker, then this stable complex measured in maximum absorption at~ 450nm. Trolox was used as a standard to generate a reference curve to compare those readings obtained by the samples. Final data expressed as micro Mollar copper reducing equivalents or in micro Mollar Trolox equivalents.

#### Study Protocol and Ethics

Study protocol approved by local ethical committee in college of science/ Babylon university, all principals which depend in this committee are according of the principles of declaration Helsinki.

**Table 1.** Seroprevalence of *Toxoplasma gondii* infection according different infertile women groups

Infertility group	No.	percentage	Infection No.	Percentage of infection	
primary	24	16.3%	6 (24)	25%	chi-s=3.2 p value=0.3
secondary	52	35.4%	7 (52)	13%	
sub-infertility	71	48.3%	15 (71)	21%	
Total	147	100%	28 (147)	19%	

**Table 2.** Serum Testosterone concentration (µIU/mL) according Hypo-Normal-Hyper values among infertile women groups

Sample	Testosterone concentration (µIU/mL)				sig	chi-s
	Hypo	Normal	Hyper	Total		
Toxoplasmosis	3%	86%	11%	19%	0.04	4.235
primary	21%	71%	8%	16%	0.027	4.87
secondary	12%	85%	4%	35%	0.055	3.66
sub-fertility	6%	80%	14%	48%	0.09	2.85
p-value, Chi-s	P = 0.001,18.9	P = 0.802,0.997	P = 0.141, .459			

**Table 3.** Serum Testosterone concentration (µIU/mL) according Hypo-Normal-Hyper values among Toxoplasmosis infertile women group

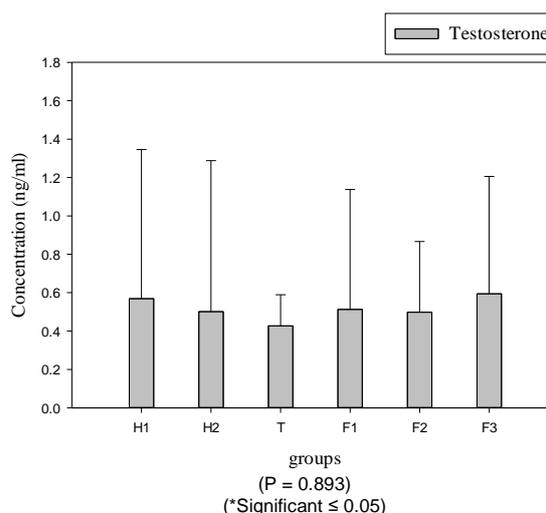
Sample	Testosterone concentration (µIU/mL)				sig	chi-s
	Hypo	Normal	Hyper	Total		
Primary	17%	83%	0%	28%	0.027	4.87
Secondary	0%	71%	29%	25%	0.055	3.66
Sub-fertility	0%	93%	7%	52%	0.09	2.85
p-value, Chi-s	P = 0.001,30.7	P = <0.4,1.6	P = 0.01, 32.6	P = <0.01, 8.8		

**Statistical Analysis**

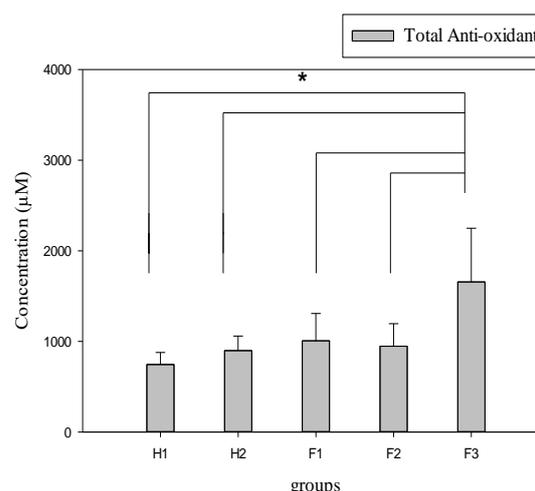
All statistical analyses were performed according statistical software program (SPSS 10 Inc., Chicago, USA). All data were checked for normality by using Shapiro-Wilk test. While Kruskal-Wallis test was performed for not-normal distributed data. One way ANOVA and Tukey post hoc analysis used in present study to detect the significant differences among groups, P<0.05 was considered as statistically significant. In addition, personal correlation and chi square used to investigate the relations among variables of our results. Variations were considered significant when P-value ≤ 0.05.

**RESULTS**

In the current study All enrolled women (147 patient and 20 control) were checked by ELIFA techniques to confirm the infection of *Toxoplasma gondii* (positive for IgG antibodies). The overall Toxoplasma infection in fertile and non-fertile women was 19% **Tables 1 and 2**. Also, Seroprevalence of Toxoplasmosis confirm that six primary infertility women (24%), seven secondary infertility women (13%) and fifteen sub infertility women(21%) were seropositive for Toxoplasma antibodies **Table 1**. Result of Testosteron concentration of subjects women are shown in **Fig. 1**. Our finding also showed no significant variation in testosterone concentration among all subjected groups.Serum TAC was higher in sub-infertile group as compare with other **Fig. 2**. In additions, serum TAC was higher (P value



**Fig. 1.** Serum Testosterone levels(ng/ml) among infected and non-infected infertile subgroups women H1= Healthy non-pregnant women, H2= Healthy pregnant women, F1= Primary infertility women, F2= Secondary infertility and F3= Sub-infertility and Toxoplasmosis group.

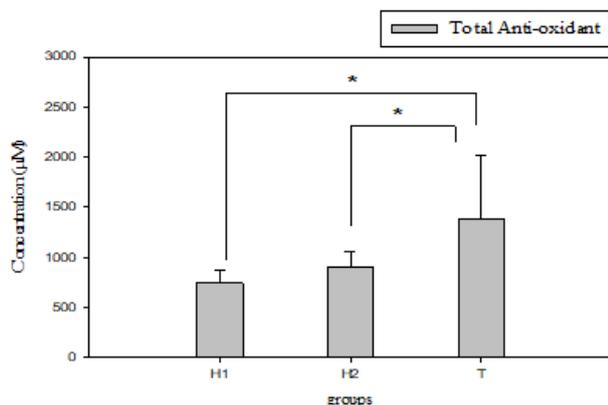


**Fig. 2.** Serum Total antioxidant capacity among healthy and infertile subgroup women

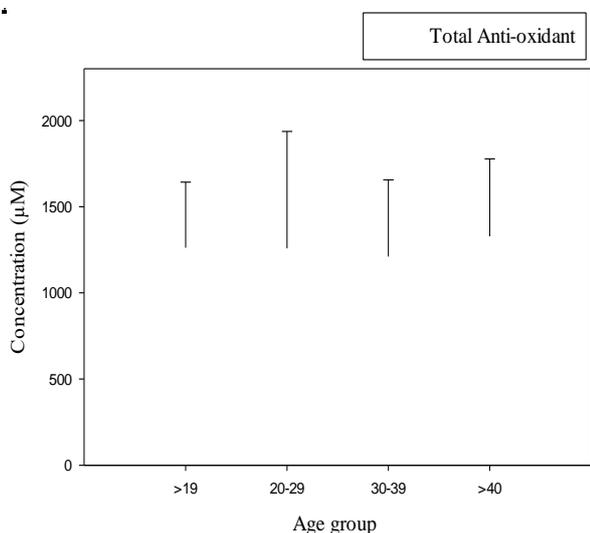
<0.05) in infected women as compare with healthy pregnant and non-pregnant female **Fig. 3**. Statistically there were no significant variation in serum TAC in different age groups **Fig. 4**. Furthermore, the correlations between intensity of infection (anti-toxoplasma IgG antibodies levels) with testosterone and TAC levels showed no significant variation (P-value=0.553,0.95 respectively) (**Fig. 5**). According to hypo-normal-hyper value as testosterone concentration, present data are shown a significant variation (P value =0.04) (**Table 3**).

**DISCUSSION**

The first studies that linked between Testosterone and changes in behavioral have their origin in experimental studies of domestic animals, and

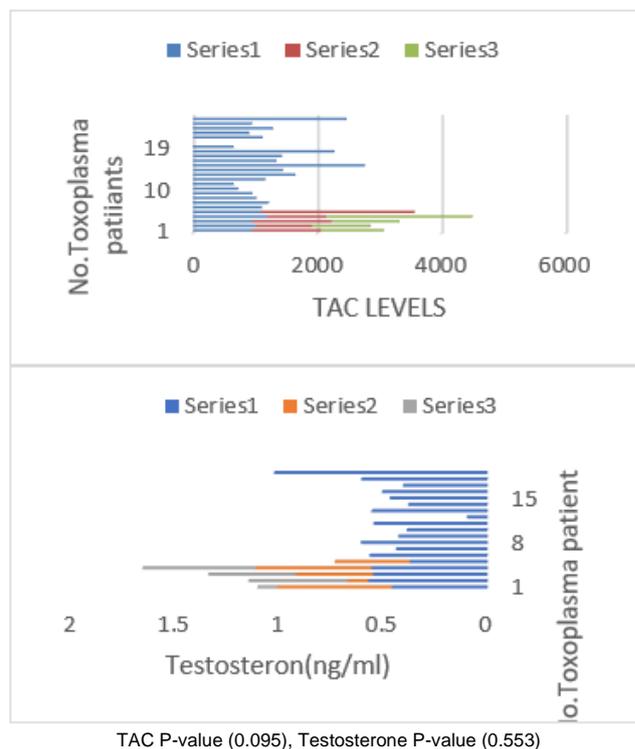


**Fig. 3.** Serum Total antioxidant capacity among infected, pregnant and non-infected pregnant women  
 F1= Primary infertility women, F2= Secondary infertility and F3= Sub-infertility and Toxoplasmosis group. H1= Healthy non-pregnant women, H2= Healthy pregnant women



**Fig. 4.** Serum Total antioxidant capacity among different age groups

experimental animal. Then followed by correlational studies with humans (Carré *et al.* 2018). These previously studies have suggested differences in the level of testosterone may be responsible for the observed behavioral changes between Toxoplasma-infected and Toxoplasma-free subjects. Increase levels of steroid hormones that have been associated with immunomodulation's (Flegr and Jaroslav 2007). Although the considerable evidence of influencing of *T. gondii* infection on production of Testosterone has been showed in different studies, but complications are still in this area (Abdoli 2014). Mechanisms most probably responsible for the observed behavioral changes in toxoplasmosis still under considerations. One of mechanisms that probably responsible for the observed behavioral changes in latent toxoplasmosis are increase male dopamine, Testosterone and hypo methylation of certain genes in amygdala infected hosts. (Flegr and



**Fig. 5.** Testosterone concentration and Total antioxidant capacity (TAC) among different levels of anti-Toxoplasma antibody (series 1=1-100, series2=101-200 and 201-300 IU/ml)

Jaroslav 2015). It's reported a significant sex differences regarding host changes in response to *T. gondii* infection (Zouei *et al.* 2018). Further, male and female personality profiles are different in opposite direction of the testosterone, this is can explain the observed gender specificity of behavioral changes in Toxoplasma parasite humans. This may be explained according present study why Testosterone does not have any considerable evidence for significant change in different case groups and in different parasite intensity (Figs. 1-5). In additions, one of explanations that we believe, the parasite shift the behavioural host by hormone manipulations just for a short period to overcome immune response, otherwise, steady state returned. Results of current study also, showed (Table 2) a significant variation in Testosterone reference range among infertile and Toxoplasmosis groups. It's not much studies for our knowledge about the association between *Toxoplasma gondii* infection and Testosterone hormone in infertile women. Interestingly, Testosterone changing have cost-benefits especially in female, where increased Testosterone may effect on immune system inhibition, whereas, the increase minimized reproductive fitness, finally decrease transmission possibilities of parasite. In opposite direction in the males where, induce immunosuppressive and maximized male reproductive fitness. The behavioral manipulation

hypothesis predicts that parasites can change host behavior in a way that benefits the parasites and not the host (Vyas and Ajai 2015). Previous studies reported that Chronic infection by *Toxoplasma gondii* induces testosterone and Hypothalamus axis activations, both of them are able to stimulate release of arginine vasopressin, a neurotransmitter associated with reproductive behavior (Tedford and McConkey 2017). In present study we have been not found statistical variations between infected and free infected women in testosterone concentration, this result disagree with the previous studies that showed significant variations in Testosterone levels and anti-Toxoplasma IgG antibody (Shahnaz *et al.* 2011, Zouei *et al.* 2018;). But according hypo-hyper value testosterone the story is different were considerable changes ( $P = 0.001$ , chi-s 18.9) especially in primary infertile women (hypo-values) and secondary infertile women (hyper-value) increased statistically ( $P = 0.01$ , chi-s 32.6). So, no correlation between changes in Testosterone and TAC in all groups (personal correlations, p-value more than 0.05). Alternatively, in an evolutionary sense, the behavioral changes induced by *T. gondii* could be side effects of the organism's increase in testosterone in order to impair the cellular immunity of the host and thus increase the chances of surviving in the host organism (Flegr and Jaroslav 2007). In addition, there were significant TAC increase in infected and subfertility groups (aborted women), this may providing us of the comprehensive picture of redox-antioxidant imbalance. An increased concentration of TAC during latent toxoplasmosis can result in different consequences, such as activations of immune cell and rapid release of ROS. This is also, a significant point, because ROS has been shown to play important roles especially enhance abortion when increase in placenta. Therefore, good point here, the gestation failure reflects successful of parasite. (Zouei *et al.* 2018). Also, its seems that different factors such as parasite strains and variations of host have different effects on the intensity of *T. gondii* infection, which consequently has diverse effects on productions of Testosterone and alteration of behavior (Mahbodfar *et al.* 2015). Based on our results

TAC increased significantly (**Figs. 2-4**) in Toxoplasmosis as compare with pregnant and non-pregnant women. Furthermore, significant variation, also seen in subfertility women as compare with others (**Fig. 2**). In Toxoplasmosis, like others human diseases, free radicals, such as superoxide radicals, hydrogen peroxide and hydroxide radicals, and other reactive oxygen species (ROS) are constantly formed in the human body (Al-Fartusie 2012). Measuring of Total antioxidant capacity (TAC) may reflect all physiological, environmental and redox status in human (Bahrami *et al.* 2016). Published data showed that balance between antioxidant-redox states have effective on mechanism of tachyzoite-bradyzoite interconversion. The current study also showed, the antioxidants in addition to reflecting the state of oxidation in the body may also be important in the process of tachyzoite-bradyzoite interconversion. Many evidences that support TAC very important for success of fertilization (Mulla *et al.* 2018). Therefore, the high level of TAC may indicate the extent of oxidative stress. Thus, another investigation needed to measure ROS, enzymatic and non-enzymatic antioxidant staff to give us an understanding picture of relatedness between infertility and activation of redox-antioxidant status in parasite free and infected women.

## CONCLUSION

Our findings have focused on the importance of a latent Toxoplasmosis infection on Total antioxidant and Testosterone levels among infertile groups women. Our data showed a weak consideration between chronic Toxoplasma infection and Testosterone levels among infertile women. Also, from our results, it could be concluded that increasing of Testosterone may consider a one of risk factors for premature termination of pregnancy especially in subfertility women.

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