



## Original Research Article

# Study the Effect of Acute Toxoplasmosis Infection on Some Hormones and the Phagocytic Activity of Neutrophils in Pregnant and Non-pregnant Women Before and After Treatment

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## ABSTRACT

This study was Included a total 90 pregnant and non pregnant women with acute toxoplasmosis were treated for 2 months with (spiramycin). Their ages ranged from 18 to 40 years. In this study the prevalence of acute toxoplasmosis were monitored in both pregnant and non pregnant women according to the presence of anti- *T. gondii* antibodies (IgM) and appeared to be 15 %, in addition to the levels of (progesterone and estrogen) hormones were measured by COBAS e411 which use Electro-chemiluminescence immunoassay (ECLIA) technique. Results showed that the acute toxoplasmosis always related with high concentration of estrogen in both treated pregnant and non-pregnant women infected with *T. gondii* which were  $(518.67 \pm 34.42)$  and  $(132.37 \pm 9.94)$  respectively when compared with non treated group. While the result of progesterone showed non- significant differences before and after treatment in both pregnant and non pregnant infected women when compared with control group. whereas the result of phagocytic activity of neutrophil by using nitro-blue tetrazolium (NBT) stain showed significant difference in the infected women comparing with treated group.

## Keywords

Toxoplasmosis,  
Progesterone,  
Estrogen,  
NBT  
stain

## Introduction

Toxoplasmosis is caused by infection with the protozoan parasite called *Toxoplasma gondii*. Acute infection in pregnant women can be transmitted to a fetus and can cause severe congenital infection result in mental retardation, blindness and epilepsy in fetus (Steven *et al.*, 2008). Congenital infection is a very serious condition with a lethal prognosis in about 10% of cases and a high proportion of disabling sequelae (Frederique, 2000). The human acquired

infection by ingestion of contaminated food by oocyst that excreted from cats faces or tissue cyst (bradyzoites) found in the meat of infected animals or from mother to fetus through placenta (congenital transmission) (Zeibig, 1997).

Toxoplasmosis is a lifelong condition but the fetus is only at risk of congenital disease when acute infection occurs in pregnancy. Although *T. gondii* infection in Healthy

subject is asymptomatic but in immunocompromised patients may have serious disease (Sitoe *et al.*, 2010).

There is evidence that *T. gondii* infection affected by sex hormones levels, as a result, can influence the immune system and thus susceptibility to disease in general and particular immunity to selective protozoa parasitic disease such as *Toxoplasma gondii*, and the importance of this parasite is due to the ability to cause congenital disease if infection occurs during pregnancy (Roberts *et al.*, 2001).

The present study showed that NBT test could be efficient and help in the determination the phase of infection whether acute or chronic because its level reflected the innate immunity which considered important in the diagnosis of the disease (Al-Dabbag and Al-Dabbag, 2006).

This study was done to review the relationship between levels of hormones (progesterone and estradiol) and acute infection by *T. gondii* in female patient Focusing on the hormone changing before and after treatment; and evaluate the phagocytic activity by Nitro-blue tetrazolium stain (N.B.T) before and after treatment and to compare the results with control group.

ECLIA technique this technology provides superior analytical performance and increased sensitivity means that it can be detected extremely low levels of antigen, as well as slight changes in levels ([http://www.roche.hu/content/dam/internet/corporate/roche/hu\\_HU/docs/cobas\\_e\\_411\\_EN.pdf](http://www.roche.hu/content/dam/internet/corporate/roche/hu_HU/docs/cobas_e_411_EN.pdf)).

## **Materials and Methods**

This study was carried on 90 pregnant and

non pregnant women infected with acute toxoplasmosis & treated for 2 months with (spiramycin) attending alkut maternity hospital and Al kut medical laboratory in Wasit province between September and May 2015. Their ages ranged from 18–40 years, all pregnant women were in the 1st to 3th initial months of their pregnancy period.

Sixty five patients with positive anti-toxoplasma IgM antibodies all screened for the presence of IgM antibodies by using COBASe411, and the other 25 sero negative IgM women were considered as control group for comparison the results. Two blood samples were taken from each woman one before treatment and the other after treatment. The sample before treatment (5ml) divided into portions, about (1ml) used to evaluate anti- *Toxoplasma* IgM via ECLIA technique by using COBAS e411, and (1ml) used to evaluate phagocytic activity by using NBT stain, and the remaining blood separated by centrifuge by blood centrifugation at 3000 rpm for 5 minutes and stored at -20 C until needed for evaluation the hormones (estrogen and progesterone) also by COBASe411.

After about 2 months treatment another blood sample (5ml) are taken from patients who were sero positive IgM, 1ml for detection phagocytic activity of neutrophils, and the remaining centrifuged to separate the serum for hormonal analysis. Kit for detection of IgM antibodies against *T. gondii* antigens in serum from (Roche Diagnostics GmbH, Mannheim, Germany) was used according to manufacturer's instructions. The test kit was used from (Roche, Germany) & COBASe411 used for the quantitative determination of estradiol (E2) and progesterone (prog) concentration in human sera.

The determination of phagocytic activity of

neutrophil by using nitro-blue tetrazolium (NBT) test; in this test neutrophils ingest the dye, nitroblue tetrazolium, and in the presence of reactive oxygen species, the yellow colored NBT compound is converted to the purple-blue formazan compound. The NBT test was performed within one hour after specimen collection, according to method of Park *et al.* (1968) with some modification. NBT test is a simple method that helpful in the diagnosis as well as management of infection (Frederique, 2000). The percentage of neutrophils that contain purple blue deposits (formazan) represents the phagocytic activity of the neutrophils.

**Statistical Analysis**

The Statistical Analysis System- SAS (2012) was used to effect of different factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study (SAS, 2012).

**Result and Discussion**

The changes in the hormonal levels in pregnant women infected with acute toxoplasmosis before and after spiramycin treatment were shown in table 1.

The result of estrogen (E<sub>2</sub>) revealed significant increased (P<0.05) in the serum level of before treatment group (518.67 ± 34.42 pg/ml) compared with healthy control (392.31± 31.80 pg/ml). While significant reduction in after treatment group (190.34 ± 19.02 pg/ml) when compared with control and before treatment groups.

As motioned in table 1 the mean concentration of progesterone showed that there is no statistical difference between all groups (P>0.05). While the changes in the hormonal levels in (non pregnant) women infected with acute toxoplasmosis before and after spiramycin treatment as showed in table 2.

The result of non pregnant infected women with acute toxoplasmosis showed significant increased (p<0.05) in the mean concentration of estrogen (E<sub>2</sub>) before treatment (123.37 ± 9.94 pg/ml) comparing with control group (51.26 ±7.77 pg/ml) and after treatment group (80.45± 5.39 pg/ml).While the mean estrogen level also showed significant increased in after treatment (p<0.05) in comparing with control.

**Table.1** The mean concentration of hormones (progesterone, estrogen) in the pregnant women infected with (Acute) toxoplasmosis before and after using spiramycin comparing with control group

parameter	Groups of pregnant patients n=43				
	Mean ± SE			LSD	P-value
	Control subject	Before treatment	After treatment		
Estrogen (pg/ml)	392.31 ± 31.80 B	518.67 ± 34.42 A	190.34 ± 19.02 C	105.65 **	0.0001
Progestron (pg/ml)	25.68 ± 3.75 A	22.82 ± 1.88 A	22.67 ± 1.46 A	6.750 NS	0.715

Values with (A, B, C) superscript in the same group represent significance differences; \*\* represent highly significant; NS represent Non-significant difference between groups.

**Table.2** The mean concentration of hormones (progesterone, estrogen) in the sera of non pregnant women infected with (Acute) toxoplasmosis before and after treatment comparing with control group

Parameters	Groups of non pregnant patients Mean ± SE				
	Control	Before treatment	After treatment	LSD	p-value
Estrogen (pg/ml)	51.26 ± 7.77 C	132.37 ± 9.94 A	80.4 ± 5.39 B	25.687**	0.0001
Progesterone (pg/ml)	3.65 ± 1.80 A	2.78 ± 1.01 A	2.77 ± 0.35 A	2.943 NS	0.829

Values with (A, B, C) superscript in the same group represent significance differences; \*\* represent highly significant; NS represent Non-significant difference between groups.

**Table.3** Phagocytic activity of neutrophil by using nitroblue tetrazolium stain in group of pregnant women infected by acute toxoplasmosis before and after treatment

NBT %	Groups of pregnant patients Mean ± SE				
	Control	Before treatment	After treatment	LSD	P-value
	10.54% ± 0.99 B	14.88 % ± 0.46 A	11.53% ± 0.41 B	1.750 **	0.0001

**Table.4** Phagocytic activity of neutrophil by using nitroblue tetrazolium stain in group of (non pregnant) women infected by acute toxoplasmosis before and after treatment

NBT (%)	Groups of non pregnant patients Mean ± SE				
	Control	Before treatment	After treatment	LSD	P-value
	8.63% ± 0.57 C	14.12% ± 0.71 A	11.82% ± 0.65 B	2.115 **	0.0001

The result of present data showed that mean concentration of progesterone was not differ significantly between all groups of the present study ( $p > 0.05$ ). The result of phagocytic activity of neutrophils by using nitro blue tetrazolium stain (NBT) in pregnant women infected with acute toxoplasmosis showed significant increased ( $p < 0.05$ ) in pre-treatment group ( $14.88 \pm 0.46$ ) comparing with control group ( $10.45 \pm 0.99$ ) and post treatment group ( $11.53 \pm 0.41$ ), but non - significant difference between post treatment and control group (Table 3).

Table 4 showed the effect of acute toxoplasmosis infection on the phagocytic

activity of neutrophils in non pregnant women before and after treatment.

The results in table 4 demonstrate significant increased ( $p < 0.05$ ) in the mean of active neutrophils in before treatment of non pregnant infected women with acute toxoplasmosis ( $14.12 \pm 0.71$ ) compared with control group ( $8.63 \pm 0.57$ ) and after treatment ( $11.82 \pm 0.65$ ), also the treated group showed significant increased than control group.

Host hormones can affect responses to infection; also parasites can have distinct effects on hormone signalling within the host. Additional studies suggest that

protozoan parasites can alter hormone concentrations in their hosts (Klein, 2004). The results of estrogen show significant increase ( $p < 0.05$ ) in pregnant women infected with acute *T. gondii* before treatment when compared with control and post treated group (Table 1). This result was agree with (Al-Warid *et al.*, 2012) who found high level of estrogen in infected women with *T. gondii* compared with non infected women.

Also agree with Taher and Abdullah (2012) who found the same result, but Omima *et al.* (2015) showed a significant decreased in serum estrogen hormone in aborted women infected with *T. gondii*, this result was seen after chronic infection. While the present study include patients in the acute phase of *Toxoplasma* infection.

The result of the current study in non pregnant infected women showed significant increase ( $p < 0.05$ ) in estrogen level in before treated group compared with after treatment and control group, table 2. This finding do not agree with Omima *et al.* (2015) who reported that there is significant decreased in serum estrogen hormone in aborted women infected with *T. gondii* compared to control group.

This findings may be attributed to that women are subjected to a regular cyclic changes in hormone level ( $E_2$  & Progesterone), these changes occurs mostly as a physiological fluctuation during menstrual cycle, these hormones has a large effects on cells involved in immune system and as a sequence of this effect on immune system will increase susceptibility to *Toxoplasma* infection (Roberts *et al.*, 2001).

The result of progesterone revealed non-significant variation between the study group in pregnant infected women (Table 1),

also Al-Warid *et al.* (2012) found no significant difference in progesterone levels between infected pregnant women and non infected.

In addition, our result agreed with Kadhim and AL-Awadi (2013) who reported that there is no significant difference in progesterone level in first trimester m but disagree with the finding that concerning the women in 2<sup>nd</sup> trimester in which there is significant decrease in progesterone level in seropositive IgG women, also with women in the 3<sup>rd</sup> trimester of pregnancy in which there is significant increase in level of progesterone in seropositive IgG, this may be explain our finding which include patients with seropositive IgM.

The result of progesterone level in the groups of study with non pregnant women showed that there were no significant difference in progesterone levels between infected and non infected women with acute *Toxoplasma* infection m although low progesterone level were noticed in infected women compared with higher level in non infected women (control group) ( $2.78 \pm 1.01$  pg/ml) ( $3.65 \pm 1.80$  pg/ml) respectively (Table 2).

Progesterone can have both stimulatory and suppressive effect on the immune system, but it is typically regarded as immunosuppressive (Kadhim and AL-Awadi, 2013), in our study it seems that the high progesterone level in non infected have immunostimulatory effect characterized by higher cellular immunity (Klein, 2004) which can overcome the *T. gondii* proliferation.

The results of phagocytic activity of neutrophils by using nitroblue tetrazolium (NBT) stain in both pregnant and non-pregnant women revealed significant

increase of NBT positive neutrophils count in infected women with *T. gondii* before treatment than after treatment and control group (Tables 3 and 4).

The efficiency of neutrophils as phagocytes is measured by nitro-blue tetrazolium (NBT) test (Gupta and Steigewald, 1974). The nitro-blue tetrazolium dye is converted by the reduction occurring during phagocytosis to an insoluble blue-black formazane deposit (Gupta and Steigewald, 1974; Park *et al.*, 1968).

In normal individuals (control group) the percentages of NBT positive neutrophils (with formazane deposits) was reported to vary between 3% and 11 % (Park *et al.*, 1968; Gupta and Steigewald, 1974). An increase in the positive NBT% was reported in the majority of bacterial and parasitic infections (Park *et al.*, 1968; Gupta and Steigewald, 1974) and may reach up to 75% in acute phase of infection and then decline gradually until return to normal values within 4–6 weeks (Roitt *et al.*, 2001). Neutrophils have an important role in the non-specific (innate) immunity of the body (Roitt *et al.*, 2001), its increase means that this kind of immunity is efficient.

The present results are basically in agreement with results of Ismail *et al.* (2013), which showed that significant increase in phagocytic activity of polymorph nuclear neutrophils (PMNs) for reduction of NBT stain.

The present study showed that NBT test could be efficient and help in the determination the phase of infection whether acute or chronic because its level reflected the innate immunity which considered important in the diagnosis of the disease, also NBT test could be used as follow up test to evaluate the degree of treatment

efficiency and patients compliance (Al-Dabbag and Al-Dabbag, 2006).

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