

## Studying the Role of IFN- $\gamma$ , Vitamin C, SOD, LH, FSH, APA and ACA in *Toxoplasma gondii* Infected Miscarriage Women

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### Abstract:

This study is designed to detect the level of cytokine IFN- $\gamma$  concentration, and some antioxidants, including super oxide dismutase (SOD) and Vitamin C, and to estimate the level of sex hormones (FSH and LH), and to determine auto-antibodies (antiphospholipid antibodies (APA) IgG/IgM, and anticardiolipin antibodies (ACA) IgG/IgM) and to estimate the blood parameters in 51 miscarriage women infected with *T.gondii* distributed depending on the type of antibodies. Additionally, 39 volunteers non-infected with *T.gondii* included (19 miscarriage women, 10 pregnant women and 10 non-pregnant women). ELISA and spectrophotometer method were used in this study. The results of IFN- $\gamma$  showed a significant increase ( $p < 0.05$ ) in the level of IFN- $\gamma$  in women infected with *T.gondii* compared with volunteer groups. The current study shows a significant difference in the level of hormones, where the concentration of LH hormone increased in married non-pregnant women compared with other groups, while the concentration of FSH hormone increased in toxoplasmosis women compared with volunteer groups. The results of antioxidants showed no significant difference in Vitamin C activity in infected women compared with volunteer groups, while super oxide dismutase (SOD) activity was significantly lower in infected women compared with volunteer groups. The results of autoantibodies showed a significant difference in anticardiolipin antibodies (ACA) IgM level, the highest level was in aborted women infected with *acute* infection (IgM Ab), while the anticardiolipin antibodies (ACA) IgG level recorded the highest level in aborted women infected with both acute and chronic infection (IgG&IgM). The results showed no significant difference in antiphospholipid antibodies (APA) IgM level in infected women when compared to the volunteer groups, while the results showed significant difference in antiphospholipid antibodies (APA) IgG level, where the highest concentration was in the volunteer groups (pregnant and non-pregnant married women). The results of blood parameters showed significant differences in differential leucocytes except for Basophils and Monocytes, and the results showed significant differences in differential erythrocytes, HB, PLT, and PCV.

**Key words:** ACA, APA, FSH, IFN-  $\gamma$ , Immunoglobulin, LH, SOD, Toxoplasmosis, Vitamin C.

### Introduction:

*T.gondii* is an important zoonotic parasite with a **worldwide distribution** and it is one of the most widespread infectious factors in Iraq (1). Human beings grow infected after birth by eating or drinking something polluted with oocysts, consuming tissue cysts of undercooked meat, or by unintentionally consuming the oocysts of the environment (2). Also, *T.gondii* can cross the placenta from mother to fetus (3). Infection with *T.gondii* during pregnancy can cause dangerous consequences in the fetues such as ocular damage,

stillbirth and hydrocephalus (4).

Unfortunately, no vaccine can stop the infection by this parasite, but only a few number of effective and active medicines to cure severe *T.gondii* infections, and none is able to remove tissue cysts (5, 6). To resist and govern toxoplasmosis, a mixture of antifolates like pyrimethamine and sulfadiazine is utilized to be the first-chosen medicines in almost all clinical situations (7). But these medicines are with significant side effects such as a reduction in platelets, red blood cells, and white blood cells (8,9). When the host is infected with *Toxoplasma gondii*, immune response occurs against *T.gondii*, leading to the production of cytokines. Dendritic and macrophages cells activate T cells inducing the

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development of CD8 T cells and CD4 Th1 cells responsible for IFN- $\gamma$  which plays an important role in the suppression of parasite growth(10). Also, some sexual hormones increase the capability to Toxoplasmosis infection, because the acute infection of *T.gondii* is associated with diversity in levels of sexual hormones (11). In addition, an imbalance between oxidative and antioxidant can lead to serious complications on pregnant women such as abortion, because the pregnancy is characterized by low defense against prooxidizing agents (12). The aim of the study is to determine the levels of cytokine IFN- $\gamma$ , some antioxidant, sexual hormones, autoantibodies and blood parameters in *T.gondii* infected miscarriage women.

## Materials and Methods:

### Studied groups

The current study was conducted in the Department of Obstetrics and Gynecology at Al-Yarmouk Teaching Hospital and the Medical City Hospital in Baghdad, from November 2016 till March 2017.

The current study included 90 women (aged between 15 to 40 years old) including women with spontaneous abortion with toxoplasmosis, aborted women without infection, pregnant and non-pregnant women without infection.

### Samples collection

Five ml of a sample of intravenous blood was taken from patient, volunteers (control) using disposable syringes. 1ml of blood was added to EDTA tube for the hematological test which involves the determination of blood parameters by Nihon Kohden Blood Cell Counter\ Japan, then the remaining 4ml are centrifuged at 5000 round per minute for about 5 minutes and kept at -20 °C for subsequent immune and hormonal analysis. ELISA assay for quantitative analysis was used for detecting the IFN-  $\gamma$  concentration by using kit supplied from Chinese company (SHANGHAI). ALPCO analyzer was used to determine quantitatively the concentration of FSH in human sera, and detecting the LH concentration was done by using kit supplied from German company (HUMAN). SOD and vitamin C activities were measured by specterophotometry technique. Vitamin C in serum was determined according to the method by Omaye *et al.* (13). Super oxide dismutase (SOD) activity was determined in serum by using the manual preparation kit \ Iraq.

### Statistical analysis

Data of various parameters were expressed by mean  $\pm$  SE and analyzed using computerized software program SPSS version 23 using one-way

Anova analysis and Duncan test. Differences were considered to be significant at ( $p < 0.05$ ).

## Results and Discussion:

### Serum level of interferon gamma (IFN- $\gamma$ ):

This study showed a significant increase of IFN- $\gamma$  level in women infected with *T.gondii* when compared to volunteer groups, as shown in Table (1).

**Table 1. The mean concentration of the cytokine IFN- $\gamma$  in the infected women and volunteers.**

Groups	N.	Mean (pg/ml) $\pm$ SE
Pregnant (non-infected)	10	11.626 $\pm$ 2.6836 <sup>d</sup>
Non-pregnant (non-infected)	10	25.298 $\pm$ 4.7515 <sup>c</sup>
Aborted (non-infected)	19	25.282 $\pm$ 1.7779 <sup>c</sup>
Aborted <i>T.gondii</i> IgG	10	47.080 $\pm$ 4.2133 <sup>a</sup>
Aborted <i>T.gondii</i> IgM	22	32.231 $\pm$ 3.5148 <sup>bc</sup>
Aborted <i>T.gondii</i> IgG+IgM	19	42.058 $\pm$ 4.6105 <sup>ab</sup>

\*different letters: significant difference at  $P < 0.05$  between the means

The results showed the great role that interferon plays in immunity against *T.gondii*. This confirms the role of interferon in the immune response against the parasite. It works to resist the parasite and inhibit it through many mechanisms made to kill the parasite or to convert it from tachyzoite to bradyzoite by many effects (14). The production of IFN- $\gamma$  activates macrophages to produce TNF- $\alpha$  (tumor necrosis factor - alpha) synergized with IFN- $\gamma$  to initiate production NO (nitric oxide). IFN- $\gamma$  also controls other effector mechanisms that fight *T.gondii* infection, including iron deprivation, production of reactive oxygen intermediates (ROI), activation of the p47 GTPases and tryptophan starvation (15). The migration of GTPase to the PV leads to disrupt it and release the parasites from the protective vacuole (16). The acquired immunity against *T.gondii* is characterized by CD4+ and CD8+ T cell activity with IFN- $\gamma$  still being significant in resisting the parasite during both chronic stages and acute stages of the infection (17).

These results come in agreement with studies done by AL-Sray (18) and Al-Fertosi *et al.* (19) who showed higher concentration of IFN- $\gamma$  in women infected with *T.gondii* compared with the control groups, while disagreed with study done by Aldabagh *et al.*(20) who showed significant dropping in abortion women infected with *T.gondii* compared with the control group.

### Estimating serum levels of autoantibodies by using ELISA technique

#### Serum level of anticardiolipin antibodies (ACA) IgG/IgM:

The results of the current study for ACA IgG test showed a significant increase in the group of aborted women with acute infection (IgM Ab), while the group of aborted women with chronic infection (IgG Ab) was significantly lower compared with other groups. The results of current study for ACA IgM test showed that the groups of (pregnant women non-infected and aborted women with chronic infection IgG Ab) had a lower level for ACA IgM significantly compared with other groups, as shown in Table (2).

**Table 2. The mean concentration of ACA IgG/IgM in the infected women and volunteers.**

Groups	N	Mean $\pm$ SE	
		ACA IgG (mlU/ml)	ACA IgM(mlU/ml)
Pregnant (non-infected)	10	15.76 $\pm$ 2.63 <sup>ab</sup>	11.602 $\pm$ 0.96 <sup>b</sup>
Non-pregnant (non-infected)	10	18.20 $\pm$ 1.97 <sup>ab</sup>	13.84 $\pm$ 1.57 <sup>ab</sup>
Aborted (non-infected)	19	18.58 $\pm$ 1.30 <sup>ab</sup>	16.45 $\pm$ 1.16 <sup>a</sup>
Aborted <i>T.gondii</i> IgG	10	12.56 $\pm$ 1.93 <sup>b</sup>	10.02 $\pm$ 1.10 <sup>b</sup>
Aborted <i>T.gondii</i> IgM	22	20.60 $\pm$ 1.71 <sup>a</sup>	13.98 $\pm$ 2.07 <sup>ab</sup>
Aborted <i>T.gondii</i> IgG+IgM	19	17.47 $\pm$ 1.93 <sup>ab</sup>	17.25 $\pm$ 0.46 <sup>a</sup>

\*different letters: significant difference at P< 0.05 between the means

The reason for the increase of anticardiolipin in the body is because anticardiolipin is antibodies to the body's own structures (autoimmunity), this means that the body attacks itself and is not specialized against a specific disease but it enters the structure of platelets and membrane of the living cell.

The association of anticardiolipin with antibodies will form small clots of blood which in turn will prevent blood from reaching the fetus across the placenta, causing abortion, or perhaps anticardiolipin attacks platelets because it contains anticardiolipin and thus impede the process of blood clotting causing an increase in the incidence of internal bleeding during pregnancy, leading to abortion (21). Therefore, the current research results showed a significant increase in the antibodies of anticardiolipin IgG, IgM than the normal ratio of volunteers groups, especially the group of aborted women infected with IgM Ab, as well as women with acute and chronic infection (IgG&IgM). While significant increase was obvious in aborted women with acute infection (IgM Ab) for ACA IgG test. This is a natural issue for women with abortion,

where the increase is consistent with the weak immune status of pregnant women and whose suffered from abortion.

#### Serum level of antiphospholipid antibodies (APA) IgG/IgM:

The results of the current study showed no significant difference at (P>0.05) for APA IgM antibody test in women with Toxoplasmosis compared with the volunteer groups.

The results of the current study showed significant difference at (P>0.05) for APA IgG antibody test, where the results showed that the level of APA in all aborted groups (except aborted women with chronic infection IgG Ab) was higher compared with non-aborted women in volunteer groups (pregnant and non-pregnant women), as shown in Table (3).

**Table 3. The mean concentration of APA IgG/IgM in the infected women and volunteers.**

Groups	N	Mean $\pm$ SE	
		APA IgG (mlU/ml)	APA IgM (mlU/ml)
Pregnant (non-infected)	10	8.560 $\pm$ 0.35 <sup>c</sup>	12.42 $\pm$ 2.29 <sup>a</sup>
Non-pregnant (non-infected)	10	7.34 $\pm$ 0.78 <sup>c</sup>	9.29 $\pm$ 0.67 <sup>a</sup>
Aborted (non-infected)	19	13.94 $\pm$ 0.97 <sup>a</sup>	10.40 $\pm$ 1.46 <sup>a</sup>
Aborted <i>T.gondii</i> IgG	10	8.900 $\pm$ 1.87 <sup>c</sup>	10.22 $\pm$ 0.56 <sup>a</sup>
Aborted <i>T.gondii</i> IgM	22	12.93 $\pm$ 1.35 <sup>ab</sup>	9.56 $\pm$ 1.27 <sup>a</sup>
Aborted <i>T.gondii</i> IgG+IgM	19	9.41 $\pm$ 1.47 <sup>bc</sup>	11.28 $\pm$ 1.37 <sup>a</sup>

\*non-significant difference at p<0.05 between the means

The increase of APA IgG antibody test in aborted women with acute infection (IgM) and both acute and chronic infection (IgM+IgG) revealed that Toxoplasmosis can perform a role in forming these antibodies with their bad consequences for pregnancy (22). One reason is the presence of autoimmune phenomenon responsible for developing these autoantibodies due to antigen similarity between some *T. gondii* epitopes and human cell membrane antigens, which might lead to cross reactivity (23).

These results disagree with previous study done by Mohammed (24) who showed that there is no relation between the existence of anti-*Toxoplasma* and anti-phospholipids antibodies.

### Estimation serum level of hormones levels by used ELISA technique

#### Serum level of Follicle-stimulating hormone (FSH):

The present results showed that the level of FSH hormone in women infected with *T.gondii* had

a higher level when compared to the volunteer groups, as show in Table (4).

**Table 4. The mean concentration of FSH hormone in the infected women and volunteers.**

Groups	N.	Mean (mlU/ml)± SE
Pregnant (non-infected)	10	24.760±2.95 <sup>b</sup>
Non-pregnant (non-infected)	10	18.443±3.07 <sup>b</sup>
Aborted (non-infected)	19	20.221±3.78 <sup>b</sup>
Aborted <i>T.gondii</i> IgG	10	30.540±4.24 <sup>a</sup>
Aborted <i>T.gondii</i> IgM	22	31.075±4.29 <sup>a</sup>
Aborted <i>T.gondii</i> IgG+IgM	19	33.900±2.86 <sup>a</sup>

\*different letters: significant difference at P< 0.05 between the means

The reason for the high level of the hormone in infected women may be because that some sex hormones associate with immune response, that means that sex hormones in high concentration associate with low immune response, which may lead to increasing susceptibility to parasitic infection (25).

These results agree with previous study done by Al-Warid *et al.*(26) who showed pregnant women suffering from toxoplasmosis had a higher level of FSH when compared with control groups. These results disagree with other results done by Obaid *et al.* (27) who showed that no significant differences appeared between the FSH concentrations in infected and control in University Students.

#### Serum level of Luteinizing hormone (LH):

The present study showed that there were significant differences at (P>0.05) between the groups, where there was significant decrease for this hormone in the groups of aborted women with acute infection (IgM Ab) and aborted women with chronic infection (IgG Ab), while the volunteer group (pregnant women) was high, as shown in Table (5).

**Table 5. The mean concentration of LH hormones in the infected women and volunteers.**

Groups	N.	Mean (mlU/ml) ± SE
Pregnant (non-infected)	10	20.4400±3.76 <sup>ab</sup>
Non-pregnant (non-infected)	10	24.4857±3.91 <sup>a</sup>
Aborted (non-infected)	19	15.3586±3.09 <sup>abc</sup>
Aborted <i>T.gondii</i> IgG	10	8.1800±1.15 <sup>bc</sup>
Aborted <i>T.gondii</i> IgM	22	6.9050±1.50 <sup>c</sup>
Aborted <i>T.gondii</i> IgG+IgM	19	19.9650±4.44 <sup>ab</sup>

\*different letters: significant difference at P< 0.05 between the means

The high level of LH in pregnant women indicates their readiness to receive the sperms and the decrease of this hormone in aborted women is a normal case as a result of the abortion, which negatively affects the mother's readiness to produce progesterone which helps in the stabilization of the fetus.

The current results disagree with a previous study conducted by Al-Garawi (28) who showed that there was non-significant increase in LH level in pregnant women infected with *T.gondii* when compared with control groups (non-infected pregnant women). She attributed the reason to the great role played by the hormone LH in the stabilization of pregnancy. While these results are in agreement with the previous study done by Al-Warid *et al.* (26) who showed a significant decrease in the group of pregnant women infected with *Toxoplasma* compared with control group (non-pregnant and non-infected women). They attributed the reason to the main task of LH which is the process of ovulation and regulation, and has a weaker role in the process of pregnancy stabilization.

#### Blood parameters test results

##### Determining the level of PLT, PCV, HB

The current study showed a significant increase in the levels of blood tests (PLT, PCV) except (HB) in aborted women with chronic infection (IgG Ab), whereas there was a significant decrease in pregnant women non-infected with *T.gondii* for all tests.

The group of aborted women with chronic infection (IgG Ab) had clear differences with other groups of pregnant women and aborted women non-infected for PLT test. There were significant differences for pregnant women group with groups of non-pregnant women, aborted women with chronic infection (IgG Ab), and aborted with acute infection (IgM Ab) in the PCV test, while pregnant women group showed a significant decrease with the rest of the other groups, in addition to the obvious differences between all groups compared to volunteers groups (pregnant and non-pregnant women) in all blood test, as shown in Table (6).

**Table 6. The mean concentration of PLT, PCV, HB in the infected women and volunteers.**

Groups	N.	Mean $\pm$ SE		
		PLT ( $10^3/\mu\text{L}$ )	PCV (%)	HB (g/dL)
Pregnant (non-infected)	10	208.90 $\pm$ 61.36 <sup>b</sup>	35.08 $\pm$ 1.41 <sup>c</sup>	11.48 $\pm$ 0.60 <sup>b</sup>
Non-pregnant (non-infected)	10	251.30 $\pm$ 61.46 <sup>ab</sup>	39.82 $\pm$ 0.93 <sup>ab</sup>	13.34 $\pm$ 0.33 <sup>a</sup>
Aborted (non-infected)	19	211.64 $\pm$ 69.17 <sup>b</sup>	37.31 $\pm$ 1.04 <sup>abc</sup>	12.10 $\pm$ 0.50 <sup>ab</sup>
Aborted <i>T.gondii</i> IgG	10	290.80 $\pm$ 36.02 <sup>a</sup>	40.70 $\pm$ 0.60 <sup>ab</sup>	12.88 $\pm$ 0.47 <sup>ab</sup>
Aborted <i>T.gondii</i> IgM	22	237.50 $\pm$ 74.15 <sup>ab</sup>	38.78 $\pm$ 0.81 <sup>ab</sup>	12.43 $\pm$ 0.38 <sup>ab</sup>
Aborted <i>T.gondii</i> IgG+IgM	19	239.63 $\pm$ 44.04 <sup>ab</sup>	36.50 $\pm$ 1.51 <sup>bc</sup>	12.54 $\pm$ 0.63 <sup>ab</sup>

\*different letters: significant difference at  $P < 0.05$  between the means

These results indicate that these indicators are not stable as they change according to the person's health status.

The differences in the results of the blood tests have effective causes, the first is the age of the group from which the sample was taken, where all age groups were combined to derive the mean of the blood tests. Therefore, these differences emerged.

Alani (29) and Bartolomé *et al.* (30) clarified that the age has an effect on the stability of blood and serological indices. The second reason of which is the oxidative stress on pregnant women and their negative effects on the body.

Atmaca *et al.* (31) reported that stress negatively affects the blood and serological changes of pregnant women leading to their instability, and the results of Alani (29) agree with Atmaca *et al.* (31) results. Bahrami *et al.* (32) showed in his experiments with rats that the number of days and

age clearly affects blood and serological indicators and some antioxidants.

Mahmood (33) showed that there was a decrease in the group of aborted women compared with the control group for blood changes as well as liver enzymes and some Interlukins.

The volunteers group for non-pregnant women, naturally shows an increase in HB relative to the rest of the other groups due to the stable health status of this group, unlike other groups where oxidative stress and clear changes in the pathway of blood indicators were observed (34).

#### White blood cell count:

The results revealed the presence of significant differences at ( $p < 0.05$ ) in all tests except Basophils and Monocytes tests, as show in Table (7).

**Table 7. The mean concentration of WBC countin the infected women and volunteers.**

Groups	N.	Mean $\pm$ SE					
		BASO ( $10^9/\text{L}$ )	EOS ( $10^9/\text{L}$ )	MONO ( $10^9/\text{L}$ )	LYM ( $10^9/\text{L}$ )	NEU ( $10^9/\text{L}$ )	WBC ( $10^3/\mu\text{L}$ )
Pregnant (non-infected)	10	0.11 $\pm$ 0.01 <sup>a</sup>	0.09 $\pm$ 0.02 <sup>b</sup>	0.51 $\pm$ 0.05 <sup>a</sup>	2.69 $\pm$ 0.33 <sup>a</sup>	7.26 $\pm$ 0.54 <sup>a</sup>	10.92 $\pm$ 0.94 <sup>a</sup>
Non-pregnant (non-infected)	10	0.13 $\pm$ 0.06 <sup>a</sup>	0.54 $\pm$ 0.19 <sup>a</sup>	0.39 $\pm$ 0.04 <sup>a</sup>	2.54 $\pm$ 0.19 <sup>ab</sup>	2.97 $\pm$ 0.19 <sup>c</sup>	7.56 $\pm$ 0.69 <sup>c</sup>
Aborted (non-infected)	19	0.06 $\pm$ 0.01 <sup>a</sup>	0.10 $\pm$ 0.02 <sup>b</sup>	0.50 $\pm$ 0.06 <sup>a</sup>	1.95 $\pm$ 0.19 <sup>ab</sup>	6.33 $\pm$ 0.82 <sup>a</sup>	9.11 $\pm$ 0.71 <sup>abc</sup>
Aborted <i>T.gondii</i> IgG	10	0.14 $\pm$ 0.03 <sup>a</sup>	0.13 $\pm$ 0.04 <sup>b</sup>	0.54 $\pm$ 0.03 <sup>a</sup>	2.12 $\pm$ 0.23 <sup>ab</sup>	4.77 $\pm$ 0.38 <sup>ab</sup>	9.48 $\pm$ 0.34 <sup>abc</sup>
Aborted <i>T.gondii</i> IgM	22	0.06 $\pm$ 0.01 <sup>a</sup>	0.13 $\pm$ 0.02 <sup>b</sup>	0.50 $\pm$ 0.03 <sup>a</sup>	1.92 $\pm$ 0.16 <sup>b</sup>	7.32 $\pm$ 0.38 <sup>a</sup>	8.10 $\pm$ 0.67 <sup>bc</sup>
Aborted <i>T.gondii</i> IgG+IgM	19	0.06 $\pm$ 0.01 <sup>a</sup>	0.23 $\pm$ 0.06 <sup>b</sup>	0.54 $\pm$ 0.04 <sup>a</sup>	2.09 $\pm$ 0.20 <sup>ab</sup>	5.83 $\pm$ 0.67 <sup>ab</sup>	10.34 $\pm$ 0.84 <sup>ab</sup>

\*different letters: significant difference at  $P < 0.05$  between the means

The current study revealed that the abortion negatively affects in aborted women with acute infection (IgM). Where the significant decreased of Lymphocytes and the high ratio of Neutrophils in the group of women infected with acute infection (IgM) evidence of the body's mobilization to defend the mother against the parasite and try to keep the fetus.

The general level of white blood cells (WBC) in pregnant women non-infected is a sign of the body's preparation for any emergency and an increase in oxidative stress but consistent with the defense mechanism and the immune mechanism is

strong in the body in contrast to non-pregnant and non-aborted women group. The release of antioxidants and free radicals in non-pregnant women is few because no oxidative stress occurs, therefore, this group does not need a high level of WBC.

These results are in agreement with a previous study done by Al-Hadraawy and Hadi (35) on the one hand, there are significant differences for some WBC indicators whether at the general rate or in neutrophil and other white cell types, as well as in the quality of interleukins and globulins among women abortions and the healthy changes in DNA.

The varying significant differences in white blood cell types are indicative of the overlap of some other factors in the immune response and change of response pattern by type of disease infection.

Tonin *et al.* (36) explained that, there is an overlap in the action of acetylcholinesterase in the inflammatory reactions of *T.gondii* in rats with infection, in addition to the overlap of butyrylcholinesterase in acute infected of IgM. This interference affects the fluctuation of the response

according to the amount of infection, so experimental rats have suffered from the high and low ratio of white cell types especially lymphocytes.

#### Red blood cell count:

Table (8) shows a significant increase in groups of aborted women with both acute and chronic infection (IgM & IgG Abs) compared with other groups.

**Table 8. The mean concentration of RBC count in the infected women and volunteers.**

Groups	N.	Mean $\pm$ SE			
		MCHC (g/L)	MCH (pg)	MCV (fL)	RBC ( $10^6/\mu\text{L}$ )
Pregnant (non-infected)	10	32.95 $\pm$ 0.59 <sup>ab</sup>	26.44 $\pm$ 1.39 <sup>b</sup>	80.18 $\pm$ 3.17 <sup>b</sup>	4.38 $\pm$ 0.16 <sup>ab</sup>
Non-pregnant (non-infected)	10	33.49 $\pm$ 0.32 <sup>ab</sup>	27.67 $\pm$ 0.89 <sup>ab</sup>	82.60 $\pm$ 2.52 <sup>ab</sup>	4.86 $\pm$ 0.19 <sup>a</sup>
Abortion (non-infected)	19	32.71 $\pm$ 0.74 <sup>ab</sup>	29.59 $\pm$ 0.43 <sup>a</sup>	88.30 $\pm$ 1.22 <sup>a</sup>	4.08 $\pm$ 0.18 <sup>b</sup>
Aborted <i>T.gondii</i> IgG	10	32.30 $\pm$ 0.63 <sup>ab</sup>	27.32 $\pm$ 0.57 <sup>ab</sup>	88.36 $\pm$ 3.29 <sup>a</sup>	4.60 $\pm$ 0.23 <sup>ab</sup>
Aborted <i>T.gondii</i> IgM	22	32.16 $\pm$ 0.37 <sup>b</sup>	27.43 $\pm$ 0.83 <sup>ab</sup>	85.14 $\pm$ 1.93 <sup>ab</sup>	4.55 $\pm$ 0.11 <sup>a</sup>
Aborted <i>T.gondii</i> IgG+IgM	19	34.20 $\pm$ 0.54 <sup>a</sup>	29.66 $\pm$ 0.84 <sup>a</sup>	86.80 $\pm$ 1.87 <sup>ab</sup>	4.21 $\pm$ 0.16 <sup>b</sup>

\*different letters: significant difference at  $P < 0.05$  between the means

This is due to the iron deficiency in the blood and the difference in the mean concentration of hemoglobin, because of the acute and chronic infection, therefore, the fetus and the mother will need a high amount of iron and oxygen as a result of the continuous loss of them due to infection and immune alert (WBC), where MCHC was increased significantly in women infected with both acute and chronic infection (IgM & IgG Abs) compared with other groups. There was also a significant increase in MCH, which is the central hemoglobin, this analysis is an important indicator of iron content in the blood, which also gives red blood cells color, and that the height may indicate anemia due to parasitic infection. The parasitic infection will consume a large amount of oxygen accompanied by an increase in the amount of iron in a single cell because the hemoglobin in the cell is associated with oxygen to reach to all the cells of the body, so the body of mother and fetus will consume high amounts of oxygen. The parasite also consume oxygen which will lead to increase the rate of iron in one cell and in the mean total cells (MCH, MCHC), but this increase does not mean an increase in the number of RBC (37). In addition to that, the volume of MCV cells in aborted women in groups of aborted women non-infected, aborted women with chronic infection (IgG Ab) and aborted women with both acute and chronic infection (IgG&IgM Abs), has risen significantly compared to volunteers groups. This indicates that the blood cell needs a larger volume to absorb the amount of iron associated with oxygen, there were no

significant differences in RBC, while RBC level in volunteers group (non-pregnant women) increased when compared with other groups because it was non-infected and non-pregnant. This indicates that, non-pregnant women does not need an excess iron amount or greater amount of  $O^2$  because it is in a normal state.

Zakaria (38) confirmed that pregnant women have a significantly lower RBC number than pregnant women with toxoplasmosis.

The results of current study disagree with results given by Al-jorani (39) who showed that, there was generally significant decrease in the blood parameters in women infected with *T.gondii* compared to the control groups.

#### Determination some antioxidants activity by used spectrophotometer technique

#### Determination Vitamin C activity by used spectrophotometer:

The present study showed that there were no significant differences at ( $P > 0.05$ ) between the groups, but there was a non-significant increase in the group of pregnant women non-infected with *T.gondii* when compared with other groups, as show in Table (9).

**Table 9. The mean concentration vitamin C in the infected women and volunteers.**

Groups	N.	Mean (mg/dl)± SE
Pregnant (non-infected)	10	1.460 ±.1720 <sup>a</sup>
Non-pregnant (non-infected)	10	1.200±.0900 <sup>a</sup>
Aborted (non-infected)	19	1.050±.1161 <sup>a</sup>
Aborted <i>T.gondii</i> IgG	10	1.300±.0548 <sup>a</sup>
Aborted <i>T.gondii</i> IgM	22	1.275±.1114 <sup>a</sup>
Aborted <i>T.gondii</i> IgG+IgM	19	1.238±.0944 <sup>a</sup>

\*non-significant differences at  $p < 0.05$  between the means

Vitamin C is known to include a defensive impact inside the follicle as vitamin C inadequacy has been accounted for to bring about ovarian atrophy, broad follicular atresia, and untimely resumption of meiosis (40).

The current results showed there was a non-significant increase in the pregnant women, that means during pregnancy vitamin C concentration increases physiologically and perhaps vitamin C reducing the risk of pregnancy loss, because it probably plays a role in compensating for the oxidative burst during early pregnancy (41,42). In addition to that, the decrease in vitamin C in infected women may be due to the production of oxidants such as free radicals by the parasite, thus vitamin C as an antioxidant prevents the body from these roots by the oxidation itself (43). Vitamin C supplementation may reduce the dangerous of pregnancy such as anaemia, pre-eclampsia and restrict intrauterine growth (44).

Previous study done by AL-Khshab (45) showed that there was a significant decrease in vitamin C in infected women compared with control groups.

#### Determination of superoxide dismutase (SOD) activity by using spectrophotometer:

This study showed a significant low level for SOD in women infected with *T.gondii* when compared with volunteers groups, as show in Table (10).

**Table 10. The mean concentration of SOD in the infected women and volunteers.**

Groups	N.	Mean(U/ML)± SE
Pregnant (non-infected)	10	141.40±1.470 <sup>b</sup>
Non-pregnant (non-infected)	10	150.30±6.469 <sup>b</sup>
Aborted (non-infected)	19	177.86±10.957 <sup>a</sup>
Aborted <i>T.gondii</i> IgG	10	84.60±16.052 <sup>c</sup>
Aborted <i>T.gondii</i> IgM	22	64.29±7.463 <sup>c</sup>
Aborted <i>T.gondii</i> IgG+IgM	19	71.38±4.717 <sup>c</sup>

\*different letters: significant difference at  $P < 0.05$  between the means

The explanation for the decreased level of SOD in these patients may be because Hydrogen peroxide formation as a result of parasitic infection will inactivate SOD (46).

This result agrees with previous study done by Kadhim (47) who showed there was a significant decrease of SOD activities in pregnant women infected with *T.gondii* when compared with control group.

Al-Kennany (48) also reported a decrease in superoxide activity in the placenta of ewes with *Toxoplasma*, and the results suggest that, the state of oxidative stress associated with histological changes in Infected ischemia may be the main cause for abortion in infected ewes.

Atmaca (31) showed that the decreased activity of SOD observed in gerbils infected with *Toxoplasma* may be associated with increased blood toxicity and oxidative stress.

This result disagree with previous study done by AL-Khshab (45) and Jafari (49) who showed there was an increase of SOD activities in serum of infected women compared with control groups, and Jafari (49) suggested that the high antioxidant enzymes provide protection against tissue infected caused by ROS.

#### Conclusions:

- 1- IFN- $\gamma$  concentration recorded elevation in the serum of women infected with toxoplasmosis in comparison with uninfected, this affirms the role of IFN- $\gamma$  in the immune response against *T.gondii*.
- 2- SOD support the hypothesis of inactivation of free radical enzyme during toxoplasmosis infection and this caused an increase of oxidative events, while vitamin C didn't report a relationship with infection.
- 3- FSH level recorded an increase in aborted women infected with *T.gondii* compared with volunteers groups, while LH level recorded a decrease in all aborted women groups.
- 4- Anticardiolipin Abs had an important role in abortion, more than the role of anti-phospholipid Abs.
- 5- The hematological parameter showed a relationship with *T.gondii* infection.

#### Conflicts of Interest: None.

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دراسة دور  $IFN-\gamma$ ، Vitamin C، SOD، LH، FSH، APA و ACA في النساء المجهضات المصابات  
بطفيلي *Toxoplasma gondii*

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### الخلاصة:

صممت هذه الدراسة للكشف عن تركيز الحريك الخلوي  $IFN-\gamma$ ، وبعض مضادات الأكسدة (فيتامين C و SOD)، والهرمونات الجنسية (FSH و LH)، والمضادات الذاتية (IgG\IgM) Anticardiolipin antibodies، Antiphospholipid antibodies (APA) IgG\IgM (ACA) والمعايير الدمية لـ 51 امرأة مصابة بداء القطط توزعت على حسب نوع الأضداد، بالإضافة إلى 19 عينة لنساء مجهضات غير مصابات بالطفيلي و 10 عينات لنساء حاملات غير متزوجات غير حوامل وغير مصابات (أعتبرت كمجاميع متطوعين)، جميع العينات أُخترت باستخدام إختبار الإمتزاز المناعي المرتبط بالأنزيم (ELISA).

بينت النتائج ارتفاع معنوي ( $p<0.05$ ) في مستوى الحريك الخلوي  $IFN-\gamma$  في مصلى نساء المجهضات المصابات بالطفيلي مقارنة مع مجاميع المتطوعين. بالإضافة إلى وجود فروق معنوية ( $p<0.05$ ) في مستوى الهرمونات، حيث ارتفع تركيز هرمون LH في مصلى النساء المتزوجات غير الحوامل مقارنة بالمجاميع الأخرى، بينما ارتفع تركيز هرمون FSH في مجاميع النساء المصابات بالطفيلي مقارنة بمجاميع المتطوعين. وكذلك بينت النتائج عدم وجود فروق معنوية ( $p<0.05$ ) في مستوى Vitamin C في النساء المصابات وغير المصابات، بينما إنخفض مستوى SOD في النساء المصابات مقارنة بمجاميع المتطوعين.

أوضحت النتائج وجود فروق معنوية ( $p<0.05$ ) في مستوى anticardiolipin antibodies (ACA) IgM حيث ارتفع تركيزه في النساء المصابات بالإصابة الحادة (موجبة للضد IgM)، بينما ارتفع تركيز anticardiolipin antibodies (ACA) IgG في النساء المصابات بكلا الإصابتين الحادة والمزمنة (موجبة للضدين IgG+IgM). في حين بينت النتائج عدم وجود فروق معنوية ( $p<0.05$ ) في مستوى antiphospholipid antibodies (APA) IgM في النساء المصابات وغير المصابات. بينما كانت هناك فروق معنوية ( $p<0.05$ ) في مستوى antiphospholipid antibodies (APA) IgG حيث ارتفع تركيزه في مجموعتي المتطوعين (الحوامل والمتزوجات غير الحوامل).

كما ان نتائج التغيرات الدمية أظهرت وجود فروق معنوية بالنسبة للعدد التفريقي لكريات الدم البيض ماعدا الخلايا العقدية ووحيدات النوى، وأظهرت النتائج وجود فروق معنوية بالنسبة للعدد التفريقي لكريات الدم الحمر، والهيموغلوبين والصفائح الدموية و حجم الخلايا المكسدة.

الكلمات المفتاحية: ACA، APA، FSH،  $IFN-\gamma$ ، غلوبولين مناعي، LH، SOD، داء المقوسات، فيتامين C.