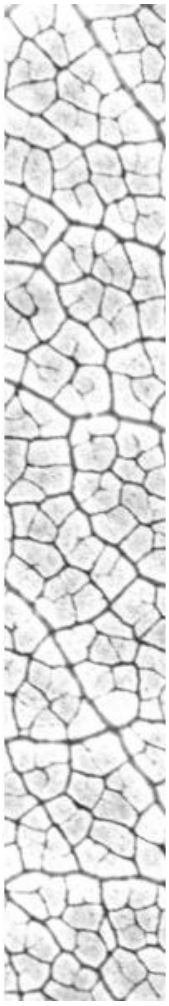

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Biochemical and hormonal study in women infected with *Toxoplasma gondii*

Balkeas A.A. Jwad [1*], Zainab A. M. Al- Haboobi [1], Nawras M. Al-Khafaji [2]

[1] Health community Department, Technical Institute of Karbala, Al Furat Al-Awsat Technical University, IRAQ
[2] College of Veterinary Medicine, Karbala University, IRAQ
*Corresponding author: inker.balk@atu.edu.iq

Abstract
Toxoplasmosis is one of the causative agents of miscarriage or birth defects in pregnant women. This study aimed to assess liver function in women with toxoplasmosis who experience repeated miscarriages. Common biochemical markers were measured to extrapolate the effects of toxoplasmosis on liver function. In total, 37 women were diagnosed with *Toxoplasma gondii* by ELISA and another 20 uninfected women were selected as a control group. The results demonstrated a dramatic increase in the serum levels of ALT and IgG in patients compared with the control group. In addition, a Pearson's Coefficient Correlation test revealed positive correlations between ALT and ALK, LH and FSH (at $p < 0.01$ for both) and patient age and LH ($p < 0.05$). Preliminary tests indicate that LH and FSH levels are lower in infected groups, but statistical significance was not established. Nevertheless, the current study clearly demonstrates that liver function is affected in patients with *Toxoplasma gondii* parasites.

Keywords: *Toxoplasma gondii*, ELISA, apportion, hormones

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INTRODUCTION

Toxoplasmosis is caused by the intracellular coccidian parasite *Toxoplasma gondii*; infecting one third of the world´s population. Infections are either acute or chronic, depending on the part of the body infected. The parasite occurs worldwide among mammals, including wild and domestic animals, with particular occurrence in members of the Felidae family, especially domestics cats (Rosso et al. 2008, Al-Warid et al. 2012, Dvorakova-Hortova et al. 2014). Infection is usually non-threatening and asymptomatic in healthy individuals, but it can cause serious congenital infections in immunosuppressive patients, such as in cases where immunosuppressive therapy is used (Montoya et al. 2000). Furthermore, during pregnancy women become more susceptible to infection, due to hormone changes, which may lead to symptom development (Robinson and Klein. 2012).

The primary method to diagnose *Toxoplasma* infection uses biomarker detection for *T. gondii*-specific antibodies, with the most important being IgG, IgM, IgA or IgE (Singh 2003). Identification of a positive titer of IgG and IgM during pregnancy in women previously negative for the anti-*Toxoplasma* IgG antibody suggests a proliferative disease condition dangerous to the fetus (Dhakal et al. 2015). Other hormones important to gestational women are the gonadotropins (LH, FSH) secreted by the pituitary basophil cells, which control the function and secretion of hormones by the ovaries. The specific actions of the gonadotropins are: LH primarily stimulates the production of hormones by the gonads and FSH stimulates the development of the germ cells (Crook 2013).

It is well known that the function of the liver is closely related to the storage and movement of dietary xenobiotics and sugars, servicing the body by detoxification processes and cross talk with other organs. Chronic toxoplasmosis can cause progressive damage to the liver, becoming more serious over time, concomitant with remarkable proliferations of the parasite population. Inevitably such damage brings about changes in the liver metabolism (Decoster et al. 1988, Hussein et al. 2015, Robinson and Klein 2012). Serum AST and ALT activities are the accepted markers of hepatocellular injury; serum ALT activity provides more specific diagnostic power than serum AST for assessing liver injury (Anya et al. 2018, Webster et al. 2013).

It has been found that specific serum enzymes have a tendency to increase after infection, which might correlate to the degree of damage of liver (Decoster et al. 1988). This liver damage cascades to a decrease in

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Table 1. Biochemical markers in *T. gondii* infected patients and control group

Transactions Grade	FSH µIU/ml	LH µIU/ml	ALT µIU/ml	AST µIU/ml	ALK µIU/ml	IgG	IgM	AGE
control	5.561 ± 0.631 A	5.508 ± 0.530 A	9.111 ± 0.629 A	10.768 ± 1.351 A	23.763 ± 9.378 A	1.015 ± 0.231 A	0.203 ± 0.075 A	30.211 ± 1.246 A
patients	3.377 ± 0.762 B	3.624 ± 4.722 A	17.607 ± 4.722 B	12.215 ± 2.018 A	83.119 ± 7.432 B	96.794 ± 13.915 B	2.710 ± 0.930 B	28.270± 0.9982 A
The level of probability	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

*A – denotes non-significance; B – denotes significance

hepatic protein synthesis (Robinson and Klein 2012). In addition, it is well established that infection with toxoplasmosis can cause round cell infiltration in the portal areas, cholestasis, swollen endothelial cells and focal necrosis of liver cells (Salman 2014). Indeed, one study observed that changes to AST and ALT correlated to the qualitative difference in severity of inflammation by strains of toxoplasma and host (Hussein et al. 2015).

Three types of toxoplasmosis prevalence were identified in Iraqi women and are diagnosed by the presence of immunoglobulin antibodies. Detection of IgM but not IgG gives diagnoses of an acute infection. Elevation to subacute level is conferred if both antibodies are detected, however chronic infection requires the absence of IgM so that the host is merely IgG positive (Oktenli et al. 2004). The aim of the current study was to detect the effect of *T. gondii* on the liver function by studying the relationship between toxoplasma infection and gonadotropin hormones, in women experiencing miscarriage diagnosed with toxoplasmosis on the basis of positive detection of antibodies (IgG and IgM) using the ELISA test.

MATERIALS AND METHODS

Study Groups

The current study focused on two groups of post-natal women (following miscarriage) who attended the teaching hospital and private medical laboratory at Karbala and Babylon provinces, from the period of 1st of November 2015 to 30th of March 2016. The first group included 37 women infected with *Toxoplasma gondii*, while the second group included 20 healthy women as the control.

Blood Collection

Five millilitres of blood were collected from patients or control group into polymer gel blood tubes with clot activator. The patient's and control group's ages ranged from 18 to 40 years. Blood samples rested in tubes following collection for 10 minutes at room temperature, then centrifuged at 3000 rpm for 5 minutes. The serum was separated and kept in sterile Eppendorf tubes at -20°C until use. ELISA was used to detect anti-Toxoplasma antibodies IgG & IgM. In addition, the LH and FSH hormones were also measured (µIU/ml). Manual techniques were used to measure the level of alkaline phosphate (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes.

Anti-toxoplasma Screening

Anti-toxoplasma was screened according to the manufacturer instruction (mention Kit and company names). The serum hormones levels LH and FSH and the alkaline phosphate were measured using Mini – VIDAS® (Human company / Germany).

Statistical Analysis

Statistical analysis was accomplished with Statistical Package for Social Sciences (SPSS). For comparison between two groups one-way ANOVA was used. The independent correlation of studied parameters was tested by linear regression analysis with the Pearson's correlation test. The significance level was set as two-tailed, and $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Results showed prevalence of toxoplasmosis including acute, sub-acute and chronic, between Iraqi women with pregnancy complications. Such findings demonstrate that due to infection with *T. gondii* ALK significantly increases, as showed in **Table 1**. Furthermore, IgG is also overexpressed in the serum of toxoplasmosis positive women compared with control group. In this group the high expression of IgG indicates chronic to subchronic infection.

In contrast, the other hormones included in this study were not so different by statistical significance measures. However, the general trend in **Table 1** indicates that the concentration of these other biomarkers may manifest more statistically robust differences when high sampling numbers are met. This is supported by other studies that have conveyed statistically lower LH measures in the same type of investigation (Al-Jowari and Hussein 2014). A similar extrapolation could possibly be made for the other hormones, FSH, AST and ALT, where a correlation analysis may help to strengthen the values given in **Table 1**.

The use of a Pearson correlation analysis gave the correlations summaries in **Table 2**. A weak positive correlation between ALK and ALT is evident, which is conveyed more clearly in **Fig. 1** ($R^2 = 0.2$). Nevertheless, this adds mild support to the argument that ALT is elevated in infected patients.

Table 2. Correlation between factors to the characteristic infected women with *Toxoplasma gondii*

		AST	ALT	LH	FSH	IgM	IgG	AGE	ALK
AST	Pearson correlation	1	.101	.014	.141	-200	.080	-266	148
	Sig (2-talled)		.552	.935	0407	.263	.598	.112	.381
	N	37	37	37	37	37	37	37	37
ALT	Pearson correlation	.101	1	.079	-.092	-.087	.306	-.052	.450**
	Sig (2-talled)	.552		.642	.589	.610	.065	.760	.005
	N	37	37	37	37	37	37	37	37
LH	Pearson correlation	.014	.079	1	.874**	-.056	-.198	.403*	.061
	Sig (2-talled)	.935	.642		.000	.740	.240	.013	.719
	N	37	37	37	37	37	37	37	37
FSH	Pearson correlation	-.141	-.092	.874**	1	.019	-.245	.357 *	.091
	Sig (2-talled)	.407	.589	.000		.909	.144	.030	.593
	N	37	37	37	37	37	37	37	37
IgM	Pearson correlation	.200	-.087	-.056	.019	1	-.367*	.090	-.027
	Sig (2-talled)	.236	.610	.740	.909		.025	.594	.874
	N	37	37	37	37	37	37	37	37
IgG	Pearson correlation	-.090	.306	-.198	-.245	-.367*	1	-.225	.0232
	Sig (2-talled)	.598	.065	.240	.144	.025		.181	.895
	N	37	37	37	37	37	37	37	37
AGE	Pearson correlation	-.266	-.052	.403 *	.357 *	.090	-.225	1	.091
	Sig (2-talled)	.112	.760	.013	.030	.594	.181		.594
	N	37	37	37	37	37	37	37	37
ALK	Pearson correlation	.148	.450**	.061	-.019	-.027	.023	-.091	1
	Sig (2-talled)	.381	.005	.719	.593	.874	.895	.594	
	N	37	37	37	37	37	37	37	37

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

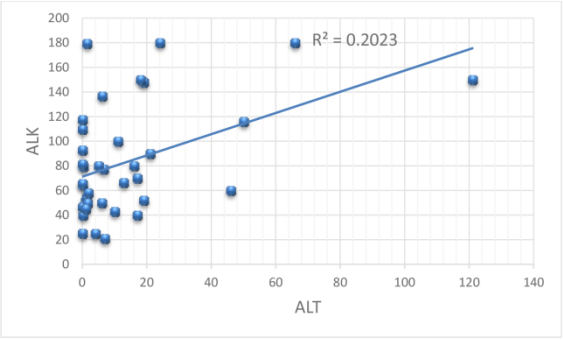

Fig. 1. The correlation between the ALK and ALT of patient infected with *T. gondii*.

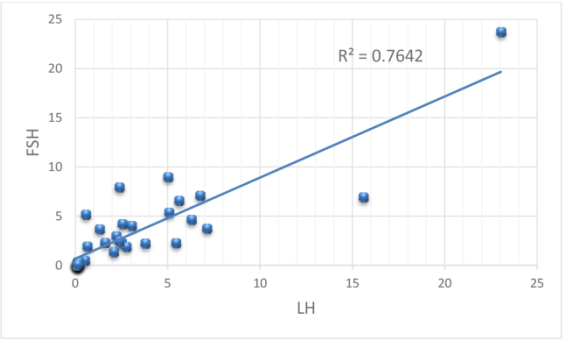

Fig. 2. The correlation between the FSH and LH of patient infected with *T. gondii*

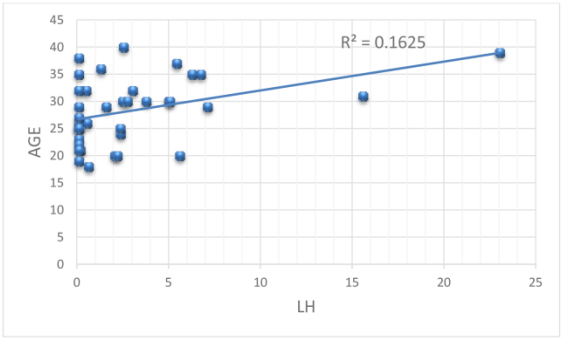

Fig. 3. The correlation between the age and LH of patient infected with *T. gondii*

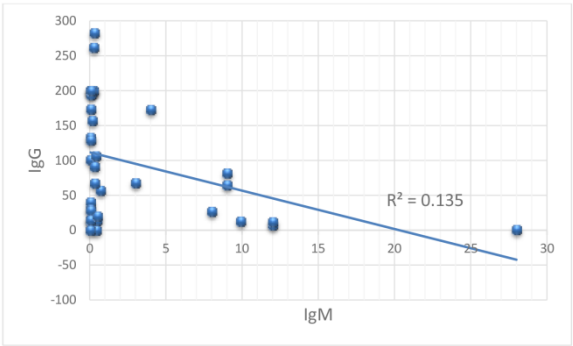

Fig. 4. The correlation between the IgG and IgM of patient infected with *T. gondii*

A stronger positive correlation was evident between the gonadotropin hormones LH and FSH, as depicted in **Fig. 2** (R^2 = 0.76), Again, despite a lack of statistical significance for LH values in **Table 1**, the strong correlation to FSH supports the observation in other studies. Yet another weak positive correlation was seen between patient Age and LH hormones, as showed in **Fig. 3** (R^2 = 0.2). Although only a mild relationship could be seen, this has revealed a possible variable that influenced the strong variability in our results in **Table 1**.

Fig. 4 depicts that there is a slight negative relationship between IgG and IgM. This is not surprising since an inverse proportionality has already been described that correlates to duration of infection, with

chronic cases having higher levels of IgG, in contrast to acute cases where IgM predominates.

Typically low concentration of some of these hormones is correlated with a weak immune response and increased susceptibility to parasitic infection (Oktenli et al. 2004). As is evident in the current study, and in other studies, *T. gondii* infection causes fluctuations in sex hormones (Roberts et al. 2001). LH hormone is secreted from the hypophysis and regulated by the hypothalamic GnRH during menstruation and post-natal development. Thus, ovary development is seriously affected with any abnormal decrease of the LH hormone level.

Due to a small sampling dataset the values in **Table 1** were not adjusted for acute and chronic cases. However, the data showing elevated levels of ALT and AST in a mostly chronic infected patient group agrees with studies of acute and subacute toxoplasmosis (Al-Jowari and Hussein 2014, Ustun et al. 2004), but again significance could not be established due to a small dataset.

Another study that dealt with acute and chronic cases separately was able to establish statistical significance for ALT increase with infection in chronic cases, but serum levels in acute cases were too variable (Portugal et al. 2004). It has been observed that specific strains of *T. gondii*, leading to differences in inflammation intensity, can significantly impact on recorded levels of ALT and AST, although the relationship of enzymatic host cell and organism has been stated in earlier studies (Al-Jowari and Hussein 2014). Nevertheless, the magnitude of increase of serum enzymes after infection might be correlated to the degree of damage to the liver (Pinon et al. 1995). Thus, ALT and AST serum level is considered a good indicator of the degree of hepatocellular damage. Furthermore, serum ALT activity is more specific than serum AST for assessing liver injury.

In conclusion, this study indicates that toxoplasmosis affects liver and kidney functions as evidenced by the significant increase in the levels of some biochemical parameters in patients group; this may possibly affect some specific enzyme systems, which can, consequently, exhibit serious pathology, including hepatitis, pneumonia, blindness and severe neurological disorders (Robert-Gangneux and Dardé 2012). Specific to the current study, chronic infection with *T. gondii* is a serious risk factor for women in pregnancy, as compared to acute cases.

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