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Toxoplasmosis and Relation with Some Immunological Markers for Aborted Women in Thi-Qar Province

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ABSTRACT

Background: *Toxoplasma gondii* is a major cause of abortion in pregnant women. Toxoplasmosis infection in the mother during pregnancy is frequently associated with transplacental transmission of the parasite to the fetus. The purpose of this study was to determine the serum level concentrations of (CD4 and IFN-)in patients with toxoplasmosis and control groups**,** aims of this study determine the serum level concentration of (CD4, IFNγ) in patients with toxoplasmosis and control groups.

and compare the different concentrations of (CD4, IFN) in acute, chronic, and control groups.

Materials and Methods From September 2020 to May 2021, a case-control study was carried out in Thi-Qar province to estimate the role of Toxoplasmosis in the occurrence of abortion among pregnant women. The current study included 120 aborted women as well as 20 healthy women as controls (Non pregnant and have no clinical history of abortion). All of these cases involved only females of reproductive age (16-44 years). Toxo-IgM and Toxo-IgG antibodies were tested first with a latex agglutination test (LAT) to detect positive samples, followed by an Enzyme Linked Immunesorbent Assay (ELISA) to detect IgG and IgM antibodies in both groups. CUSABIO method for measuring human interferon (IFN-) and 2-human cluster of differentiation 4 (CD4) (USA).

Results: IFN-γ values of a patient with Toxo- IgG, and Toxo- IgM were (31.4&38.64) respectively which is significantly higher than the control value (28.80) CD4 value in a patient with IgM was (3.045) which is significantly higher than the control patient value (2.925). Also, CD4 value of patients with Toxo IgG (4.457) was higher than control with a significant value.

Conclusion: The concentration of IFN-Y was increase in acute infection and decrease in chronic of toxoplasmosis. The concentration of CD4 were increased with chronic infection compared with acute.

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Keywords: *Toxoplasma gondii*, Abortion, IFN-γ, CD4.

INTRODUCTION

Toxoplasmosis is caused by infection with the protozoan parasite Toxoplasma gondii, which is found on all continents in humans and other animals (Dubey, 2020). This parasite has three major genotypes (types I, II, and III) that differ in pathogenicity and prevalence in humans. For example, in Europe and the United States of America (USA), the type II genotype is responsible for the majority of cases of congenital toxoplasmosis (Hussain et al., 2017). According to estimates, T. gondii infects 23% of adolescents and adults, accounting for 24% of food-borne illness deaths in the United States. Although most of these infections are asymptomatic or cause self-limited symptoms (e.g., fever, malaise, and lymphadenopathy), infections in immunocompromised people (e.g., people with acquired immunodeficiency syndrome [AIDS]) can be severe (Limon et al., 2017). Furthermore, parasite infections in pregnant women can cause serious health problems in the fetus (i.e., congenital toxoplasmosis) and severe sequelae in the infant (e.g., mental retardation, blindness, and neurological disorders). T. gondii infection is becoming more widely recognized as a problem in non-pregnant, immunocompetent adults, where acute infection can result in impaired vision (Hussain et al., 2017).

Due to the non-specificity of clinical signs of toxoplasmosis, serological test results have been paired with clinical signs evaluation in diagnosing toxoplasmosis. About 90% of immunocompetent patients have asymptomatic toxoplasmosis infection. Humoral immunity (H.I.) and cell-mediated immunity (C.M.I) will be activated against *T. gondii* that infect intracellular and may pass through extracellular space to find new host cells (Aghwan *et al.*, 2010). serum level of (CD4 & IFNγ,) in patients of toxoplasmosis, so increases or decreases during or after infection. Aims of this study Detection of T. gondii in Aborted Women in Thi-Qar Province, also to determine the serum level concentration of (CD4, IFNγ) in patients with toxoplasmosis and control groups.

and compare the different concentrations of (CD4, IFN) in acute, chronic, and control groups.

MATERIALS AND METHODS

Study design

A case-control study was conducted to estimate role of Toxoplasmosis in occurrence of abortion among pregnant women in Thi-Qar province from September 2020 to May 2021. The present study was carried out on 120 aborted women, and 20 healthy women were studied as control (Non pregnant and have no clinical history of abortion). All of these cases were limited to females only in the reproductive age (16-44 years).

Samples processing

Five milliliters of human blood were collected from each subject (patients and controls) via vein using disposable syringes and transferred to sterilized test tubes and enabled to coagulate at room temperature for 30 min, the sample was separated by centrifugation at 3000 rpm for 15 minutes to obtain serum, Measurement of Human Interferon γ (IFN-γ) and 2- Human cluster of differentiation 4(CD4) ELISA method CUSABIO (USA).

Analytical Statistics

The SPSS (Statistical Package for Science Services) version 26 and Excel 2019 were used to conduct computerized statistical analysis. Utilizing the Chi-square (X2), one way ANOVA (Continuous variables), and Probability (P) to compare (P value). P values less than 0.05 were deemed statistically significant (S), less than 0.01 were deemed highly significant (HS), while results more than 0.05 were deemed non-significant.

RESULTS

The seroprevalence of IgG and IgM anti-Toxoplasma antibodies were positive in 35/120 cases (30%) and 20/125 cases (16%) respectively, However the Overall Seroprevalence of anti- T. gondii antibodies in aborted women was 36%. In this study, mixed seropositive for IgG and IgM

were not recorded, whereas healthy women were 0% for all antibodies; the total seroprevalence of all antibodies in both groups was positive in 55/140, as shown in table 1.

As shown in Table 2 shown age group (28-33) years have the highest frequency rate (34.6%) among age groups infected with toxoplasmosis followed by an age group (34-40 years) with a rate (29.3%). The age groups (16-21 years) was the lowest frequent age groups with rates of 5.2%.

In the table 3 as shown the distribution of abortion numbers for the total study sample was shown in (Table 4-3). The highest frequency rate (36.4%) was in women with single abortion, followed by women with a history of double abortions (38.1%). Women with a history of three abortions were the lowest frequent among the total study population, with a frequency rate of 12%.

Table1. Overall Seroprevalence of anti- T. gondii antibodies in aborted and healthy women.

Study groups	N	+ve IgM	%	+ve IgG	%	+ve IgM & IgG	%	total
Aborted women	**120**	**20**	**16**	**35**	**30**	**0**	**0**	**55**
Healthy women	**20**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Total	**140**	**20**	**14**	**35**	**25**	**0**	**0**	**39**

Table 2. Distribution of Patients and control subjects according to the age groups.

	Age groups	Patients N(%)	Control N(%)
Total Study Sample	16-21 years	3(5.4)	3(15)
	22-27 years	6(11)	5(25)
	28-33 years	19(34.6)	7(35)
	34-40 years	16(29)	4(20)
	40 years>	11(20)	2
	Total	55(100)	20(100)

Table 3. Distribution of study sample according to the abortion number.

Abortion features	Descriptive	Frequency	%
Number of Abortion	Single Abortion	31	56.4
	Double abortion	18	32.7
	Multiple abortion	6	10.9
Total		55	100

The time of abortion was classified at first, second and third trimester according to time of pregnancy, the percentage of positive patients at first trimester higher than other (45.4 %), as shown in Table(4).

Table (4) Distribution of infected women in relation to (IgG-IgM) anti-Toxoplasma antibody according to abortion time.

	Descriptive	Frequency	%
Abortion Time	First Trimester	25	45.4
	Second Trimester	17	30.9
	Third Trimester	13	23.7
Total		55	100

The two groups (IgM & IgG) showed highly significant differences compared with control group as in table (5).

Table (5): Comparison of Interferon-γ of patient's groups with a control group.

Study groups	Interferon-γ(pg/ml)		
	Mean	SD	P. value
Aborted with TOXO IgM	38.64	± 6.240	**<0.001**
aborted with TOXO IgG	31.40	± 5.885	
Control	28.80	± 6.304	

SD: Standard deviation, pg: pictogram and, γ: Gamma

The two groups showed highly significant differences compared to each other and to the control group as in Table (6)

Table (6): Comparison of CD4 level of patient's groups with a control group.

Study groups	CD4 (pg/ml)		
	Mean	SD	P .value
Aborted with TOXO IgM	3.045	± 0.7527	**<0.001**
Aborted with TOXO IgG	4.457	± 2.109	
Control	2.925	± 1.457	

Aborted with TOXO IgM group show positive no significant correlation as shown in figure (1). Also, figure (1) show completely no association between the same parameters in aborted with TOXO IgG and control groups.

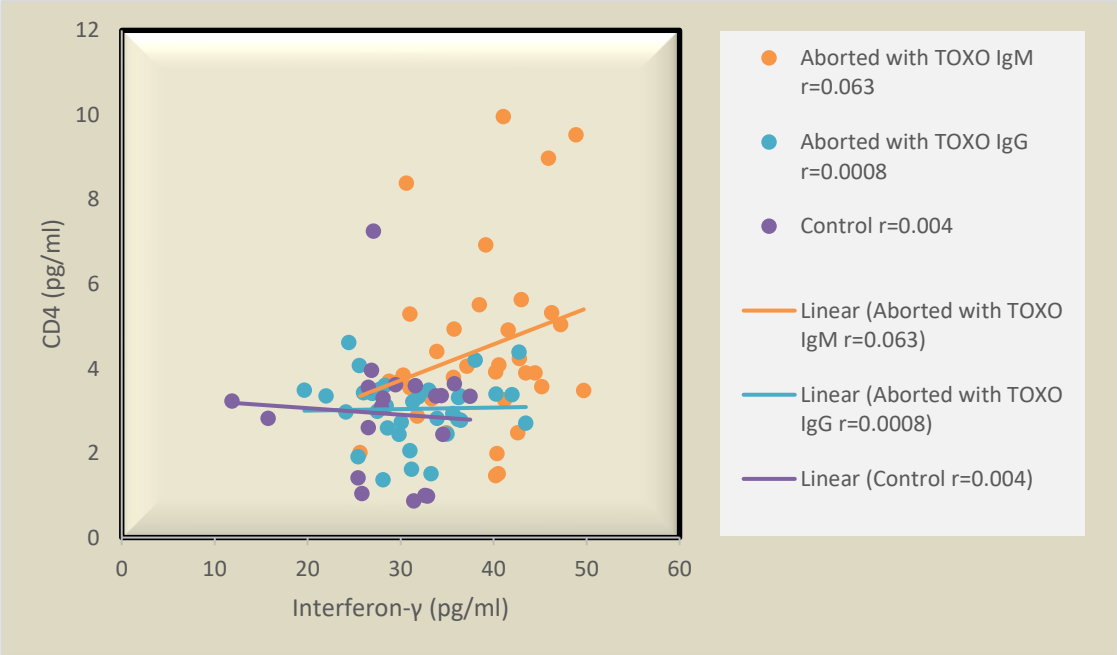

Figure (4-2): Regression analysis between interferon-γ with CD4 level for the total study population

DISCUSSION

Out of 140 samples, 55 (40%) were positive for IgG & IgM anti-Toxoplasma antibody (28% and 11% for IgG and IgM respectively in aborted women, which is similar to (Aziz et al., 2011) who found that (20.5%) women were positive for IgG and (13.9%) women were positive for IgM. Toxoplasma prevalence among pregnant women with single or multiple abortions in Salah-Adden was 26.1% for IgG and 3.1% for IgM, respectively (Al-Ddory et al., 2011). In Kirkuk, only 54 (16.9%) of 319 pregnant women tested positive for Toxoplasma gondii using IgM ELISA. These differences could be due to sample size, as well as different circumstances and times of sample collection and processing (Kadir et al., 2011).

Although the frequency of T.gondii infection has been reported to increase in older age groups in previous studies, this study found the highest rate of infection within the age group (28-33 years) and the lowest percentage of infection within the age group (16-21 years). This high rate of seroprevalence in the 28-33 age group may be due to greater contact with cats or infected things and vegetables than other age groups. This finding is similar to the findings of (Fallahi et al., 2009), who discovered a high rate of seropositivity in the 25-30 age group in Iran. While (AL-Taei et al.,2015) demonstrated in her study that the age group (28-37 years) had the highest rate of infection and the lowest percentage of injury (10-19 years). The current study found no statistically significant relationship between the percentage of infection and the number of abortions, despite finding high rates of infection among women who had a single abortion. The current study agreed with (Al-Khashab et al., 2009 & Karem et al., 2007) that the highest rate of infection was found among women who had a single abortion, but it disagreed with (Hadi et al., 2016) that there was no relationship between the injury and the number of abortions. According to (Al-Ghurairy et al., 2007), the current study found that the number of abortions is two times higher than the number of abortions performed once. The reason for the high incidence among aborted women could be a return to the type of acute injury or reactivation of chronic injury due to the pregnant mother's body's decreased immunity, as the time of the injury during pregnancy plays an important role in determining the fate of the fetus. In terms of abortion stages, a recent study found a high incidence rate of abortion in women during their first trimester of pregnancy. These outcomes High abortion rates during the first trimester of pregnancy are consistent with the findings of studies in Salah - Adden province (Al-Ddory et al., 2011) and Kut (Al-Mayahi et al., 2011). Also, (Juma et al., 2011) discovered that the highest percentage of abortions occurred in the first trimester for those who tested positive for T. gondii antigen in tissue using monoclonal antibodies. This corresponds to the fact that the degree of fetus resistance as well as the immune acquired spontaneously through the placenta, so the fetus is more vulnerable to infection during the first trimester of non-immune system is completed and is composed of opposites in the body fetus after the third month of pregnancy (Roberts et al., 2001).

When compared to the control group, the two patient groups (IgG and IgM) showed highly significant differences. During infections, interferon-gamma (IFN-gamma) has been identified as an important immune-modulating cytokine in mice and cattle. While IFN-gamma can protect cattle from abortion, high levels of IFN-gamma at the maternal-fetal interface increase fetal death. IFN-gamma is required for the acute control of T. gondii and is dependent on IFN-gamma-driven, cell-mediated immune (CMI) responses, with IFN gamma produced primarily by (IL-12)-stimulated natural killer (NK) cells and T lymphocytes (Coombs et al., 2020).

The current study discovered a significant difference (P0.001) in IFN gamma when comparing infection TOXO IgM, TOXO IgG, and control groups. This finding is consistent with the findings of Sasai et al. (2018), who discovered that antigen-presenting cells like macrophages and dendritic cells strongly activate T cells and induce the development of Th 1 cells and antigen-specific killer CD8 T cells. These T cells and Group 1 innate lymphoid cells are the primary producers of IFN-, which stimulates cell-autonomous immunity in T. gondii-aborted cells. In anti-Toxoplasma cell-autonomous immune responses, IFN-inducible effectors such as IFN-inducible GTPases, inducible nitric oxide synthase,

and indoleamine-2,3- dioxygenase play different roles in suppressing T. gondii growth and direct killing (Sasai et al., 2018). Most cells respond to IFN- stimulation by expressing hundreds of genes, including four families of GTPases: MX proteins, p47 IRGs, VLIGs, and p65 guanylate-binding proteins (GBPs) (MacMicking, 2012). IRGs and GBPs are important for IFN-γ-induced anti-*Toxoplasma* responses. This study showed a highly significant difference compared to each other and the control group agreed with the studies of (Al-Khafajy, 2004;Hafedh *et al*., 2020). The cytokine network is controlled by a set of effective immune cells especially T cells, macrophages, and dendritic cells which release two types of cross-regulatory cytokines called pro-inflammatory and anti-inflammatory cytokines. The cytokine signaling process also participates in the development of immunological memory and tolerance against invading pathogens. In Toxoplasma-aborted women, up regulation ofTh1 CD^{4+}cells which is represented by increasing TNF-α and IFN-γ concentrations reflecting protective inflammatory responses against Toxoplasma infection (Hafedh *et al*., 2020).

CONCLUSION

In this study concluded that. The concentration of IFN-Y was increase in acute infection and decrease in chronic of toxoplasmosis. The concentration of CD4 were increased with chronic infection compared with acute.

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