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RESEARCH ARTICLE

Immunological Impact of Toxoplasmosis Infection in Women Aged 21-31 in Dujail, Iraq: A Comparative Study

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ARTICLE INFO **ABSTRACT** 101 samples of toxoplasmosis parasite were collected from Dujail Hospital Received: May 21, 2024 and some private laboratories from various areas of Dujail district, for the period from July 2023 to December 2023, and infection was diagnosed Accepted: Jun 18, 2024 through TORCH analysis, and the number of samples infected with toxoplasmosis reached 66 positive samples out of 101 samples, and the ages of all were The samples were between (21-31) years old, where the **Keywords** percentage of the total amounted to 65.3%. It was found that there was a **Toxoplasmosis** relationship between women infected with toxoplasmosis and age groups, Some immunological criteria and there was a significant difference between the percentages of age groups, as age group (21-26) recorded the highest percentage of infection Iraq with toxoplasmosis, and it reached 67.2%, and age group (26-31) had the lowest percentage of infection, reached 64.1%. There was a highly significant difference of 0.01 in Cysteine 9.07±2.77 among the infected *Corresponding Author: group when compared with control group, and also a significant difference rana.mahdi32@st.tu.edu.iq of 0.05 in TNF- α 264±22.5 in infected women group compared with control group, and there were no significant differences in both, INF-Y, NK cells CSF were 104.9±26.6, 793±190, and 309.7±54.9 among the group of affected women compared with control group. The concentration of IgG Cysteine, TNF-α, INF-Y, NK cells, IgM, and CSF was measured according to age groups, and it was found that there were highly significant differences of 0.01 for Cysteine in the group of affected women when compared with control group, and significant differences of 0.05 for TNF- α . α between the group of affected women and compared with control group, and there were no significant differences for INF-Y, NK cells, CSF in group of affected women when compared with control group.

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by a protozoan intracellular parasite that infects humans and warm-blooded animals as intermediate hosts, while a number of members of feline family are considered final and intermediate hosts for the parasite (Mahmood et al., 2022). The parasite infects humans through eating food or water contaminated with egg cysts secreted in the feces of infected cats, or through eating raw or undercooked meat that contains tissue cysts, and it can be congenitally transmitted from the infected mother to the fetus via the placenta (Addo et al., 2023).

The parasite T. gondii belongs to the phylum Apicomplexa, Class: Sporozoasida, which includes a number of intracellular parasites (Hill and Dubey, 2014).

The immune system plays an important role in controlling toxoplasmosis infection through adaptive immune mechanisms. The autoimmune response stimulates two types of immune response: the cellular immune response, which appears mainly in the form of T-cells, dendritic cells, mononuclear cells, macrophages, and natural killer cells (NK cells). As for the humoral immune response, in which antibodies produced by B-cells participate, these antibodies play a role, in cooperation with complement proteins, in controlling the infection and removing the parasite (Cald and De Souza, 2018).

Immunoglobulin G (IgG) This type of immune globulin in the human body occupies about 70-75% of the total number of antibodies in the body and 15% of the total protein in the serum of adults, and its molecular weight is 150,000 Daltons. IgG is effectively transferred across the placenta, as well as colostrum and breast milk, from the mother to the fetus, and the transfer process increases in the last stage of pregnancy due to its ability to protect the newborn from some diseases during pregnancy and the first months of pregnancy and its life after birth (Piuri et al.,2019).

Immunoglobulin M (IgM) This type occupies about 5-10% of the total antibodies in blood serum and is in the form of a pentameric particle with a molecular weight of 900,000 daltons. It is one of the most effective antibodies in activating the classical pathway of the complement system, which is the first line of defense for antibodies (Racine et al., 2011).

Natural killer cells are lymphocytes that are produced under the influence of thymus gland, and they are an important type of white blood cell that responds quickly to foreign substances, and natural killer cells work to attack and kill cancer cells (Bain, 2017).

Gamma interferon INF-Y is known as immune interferon and is produced mainly by lymphocytes located between epithelial cells when stimulated, which in turn activates natural killer cells, cytotoxic T-lymophocytes, macrophages, and neutrophils to kill The nutritional phase. Interferon-gamma also plays an important role in converting the phase from the rapidly reproducing tachyzoite form found in cases of acute infection to the slow-reproducing bradyozite phase found in chronic cases of infection (Bohne et al. 1993), then suppressing the reverse transformation (converting from the slow phase to the rapid phase) in chronic infection (Jones et al., 1986).

TNF- α Tumor necrosis factor is a potent, multi-active cytokine that causes a wide range of effects on a wide range of cells. It has been shown to affect hormone synthesis, placenta formation, fetal development, and steroid formation. It has an important role in placental differentiation and the birth process, and at the same time its ability to terminate pregnancy has been proven (AL-Ubaidi and Shareef ,2021)

In recent years, it has become clear that TNF- α stimulates inflammatory responses not only directly by stimulating inflammatory gene expression but also indirectly, by causing cell death, stimulating inflammatory immune reactions, and disease progression. 157 Amino Acids Produced mainly from the activation of macrophages, T lymphocytes, basophils and dendritic cells, TNF-alpha has been found to be responsible for production of lammatory acute inflammatory response, which has the ability to activate the adaptive immune response against the T. gondii. It stimulates natural killer cells to secrete interferon gamma INF-Y during the acute phase of toxoplasmosis infection, and it also has a role in activating the cellular killing mechanism of macrophages of T. gondii (Sana et al, 2022).

Cysiteine is the main virulence factor of T. gondii, and recent studies have demonstrated that it has a role in pathogenesis and leads to the death of host immune cells, for example IgM and IgG, as well as mucus depletion and microbial dysbiosis, and as the main virulence factor (Allain et al., 2019).

Granulocyte-Colony Stimulating Factor (G-CSF) is a glycoprotein that stimulates the bone marrow to produce granulocytes and stem cells and release them into the bloodstream. It has a role in the life, differentiation, reproduction and function of neutrophil cells (et al., 2013)

The study aimed to investigate the extent of the prevalence of toxoplasmosis among aborted women in Dujail district, and to study the extent to which some prevalence criteria affect the infection rate, such as age groups, while evaluating the extent of the impact of toxoplasmosis infection on some immune parameters such as IgG, IgM, NKcells, INF-Y, and TNF-. α, Cysteine, G-CSF.

MATERIALS AND METHODS

Collection Samples

Samples were collected from Dujail District / Salah al-Din Governorate, randomly from pregnant women and miscarriages who arrived at the government hospital and some private medical clinics, whose ages ranged between 20 to 31 years, during a period of 6 months. The number of samples collected was 101 blood samples. From women suspected of being infected with the parasite, blood was drawn from a vein using a sterile medical syringe with a capacity of 5 ml, then it was placed in gel tubes and the serum was separated from them using a centrifuge at a speed of 300 rpm for 10 minutes. Then the serum was placed in Eppendorff tubes and the serum was divided. They were divided into several sections in the plastic tubes, and information was fixed on them, such as: name and age, and then the samples were frozen at a temperature of (-20°C) until the tests under study (concentration of specific antibodies IgG, IgM) and the value of immune indicators (NK cells, INF-Y, TNF- α , G-CSF, Cestine).

Immunological tests

Estimation of IgG concentration: The IgG concentration was estimated using the analysis kit (Kit) produced by the German company Mindray.

Estimation of IgM concentration: The concentration of IgG was estimated using an analysis kit produced by the German company Mindray.

Estimating the concentration of killer cells (NK cells): The concentration of NK cells was estimated using an analysis kit produced by the Chinese company Sunlong.

Estimation of the concentration of interferon gamma INF-Y: The concentration of (INF-Y) was estimated using the analysis kit (Kit) produced by the Chinese company Sunlong.

Estimation of the concentration of $(\alpha$ -TNF) necrosis factor Tumor: The concentration of $(\alpha$ -TNF) was estimated using an analysis kit (Kit) produced by the Chinese company Sunlong.

Estimating the concentration of cysteine in serum: The concentration of cysteine was estimated using the analysis kit (Kit) produced by the Chinese company Sunlong.

Estimation of the G-CSF factor or CSF3 (Granulocyte-Colony Stimulating Factor): The concentration of G-CSF was estimated using the analysis kit (Kit) produced by the Chinese company Sunlong.

Statistical analysis: The results were analyzed statistically by applying the Minitab statistical program. Ver 17. According to the t-test and the (ANOVA) F test. The arithmetic means were compared to determine the significant differences using the Duncan multinomial test with a probability level of 0.05.

RESULTS AND DISCUSSION

101 samples of the toxoplasmosis parasite were collected from Dujail Hospital and some private laboratories from various areas of Dujail district. The period of collecting the samples was six months from the beginning of July 2023 to December 2023, when the parasite was diagnosed through TORCH analysis, and the number of samples infected with toxoplasmosis reached 66 positive samples from Out of 101 samples, the ages of all samples were limited to (21-31) years, with the percentage of the total amounting to 65.3%, as shown in Table (1).

The reason for the appearance of a high percentage in our current study on toxoplasmosis may be that most of the women visited hospitals and private clinics for the purpose of care during pregnancy and to ensure that they were not infected with diseases that put the mother and her fetus at risk. We find that the incidence of toxoplasmosis is constantly increasing year after year. Others, especially in summer compared to the winter due to seasonal changes. In the hot months of the year, it helps to revive the parasite cysts that cause infection and its persistence for a longer period. The reason for the increase is also due to the large number of stray cats, as well as raising cats in homes, as well as the low level of health education, food contamination and lack of Washing knives well after cutting meat, ignorance of the source of infection or trying to avoid it, or the lack of clear signs of those infected or so-called subclinical signs, which are considered one of the important factors in the spread of parasite infection due to the difficulty of diagnosing it in the early stages beyond conducting the necessary clinical examinations.

The results of the current study showed that there is a relationship between women infected with toxoplasmosis and age groups, and there was a significant difference between the percentages of age groups, where age group (21-26) recorded the highest percentage of infection with toxoplasmosis, and the percentage reached 67.2%, and the age group (26-31) The lowest infection rate was 64.1%, Table (2).

The results of the current study showed that there were slight significant differences between the age groups of those infected, and when comparing this study with previous studies in Iraq, we found that it differed with the study of Saleh (2011), who indicated that the highest incidence of toxoplasmosis is the age group (30-39). The reason for infection of young women may be that they are heavily involved in housework, especially dealing with meat, preparing salads, cooking, and cleaning, and this is a reason for their exposure to risk factors for toxoplasmosis and other diseases (Molan and Rasheed, 2016). The reason for this variation may be the difference in environmental conditions, the immune status of the infected people, social and cultural differences, the age of the host, and the terrain.

The current study showed that there were highly significant differences for IgM and IgG with a ratio of 0.01and it was 1.872 ± 0.472 , 42.70 ± 7.011 between the infected group when compared to the control group. With highly significant difference of 0.01 in Cysteine 9.07 ± 2.77 among the infected group when compared with control group, and also a significant difference of 0.05 in TNF- α 264 ± 22.5 in the group of infected women compared with the control group, and there were no significant differences in all of, INF-Y, NK cells CSF were 104.9 ± 26.6 , 793 ± 190 , and 309.7 ± 54.9 among the group of affected women compared with the control group, as shown in Table (3).

The results of the current study showed that there were significant differences and high percentages for both IgG and IgM, and the results of this study agreed with Obaid Alajeeli (2022) in Babylon, where they recorded the highest infection rate among people with toxoplasmosis with the IgM antibody.

IgG begins to appear during the second or third week, and its level gradually rises, then begins to decline after that, and remains at low levels for long periods that continue throughout life (Remington and Macleod, 1981). As for IgM, it begins to rise two weeks after infection, which is an indicator of the onset of acute infection. It begins with the onset of the first week of infection, and its highest levels continue in the second week, then it begins to gradually decrease, and levels remain low for a period of more than six months (Dubey, 1986).

Variation in immunoglobulin levels may be due to several factors, such as the number of samples, type of test method, geographical location of the study, and infection with other bacterial diseases (Salman, 2007). The reason for the increase in IgG and IgM in blood serum of women infected with parasite is the tendency of the antibody to remain for a period Long in cases of infection, antibodies

represent an important defensive role against the antigens of the rapid reproducing phase, Tachyzoite, and the slow reproducing phase, Bradyzoite, in order to control the infection due to the ability of antibodies to reduce the number of phases formed (Adler, 2015).

One of the reasons for the high infection rate is that some studies have shown that it may be due to the large number of stray cats in the governorate, eating undercooked meat, and not washing fruits and vegetables well, which are the main source of transmission of disease. In addition to the lack of health and cultural awareness and the lack of sufficient information about how to deal with pets, such as cats, and raising them inside homes, and the dangers of allowing them to roam and stay inside homes for a long time, which increases the possibility of their exposure to surfaces contaminated with their waste, which contains one of the infectious stages of the parasite (AL-Shahwan et al., 1996).

As for killer cells (NK cells), there were no significant differences, as the results of the current study agreed with a study conducted by (Afifa et al., 2017) and her group in Sweden, which showed that it is possible for the parasite to infect any type of cell, including cells from the immune system, and Including killer cells, NK cells, that is, they become a parasitic cell and sabotage the functions of NK cells, such as not producing cytokines, including INF-Y. This is beneficial to the parasite, and this is due to the reason why INF-Y was not also increased in our current study. It differed with (Christopher et al., 1997) which recorded highly significant differences.

As for INF-Y, there were no significant differences that agreed with the study (Afifa et al., 2017) in Sweden, and disagreed with the study (2005, AL-Sorchee) and (Abdollah et al. 2011). It is possible that the reason for its not increasing is that production Excessive INF-Y and an uncontrolled response lead to disease in the host, or as a study conducted by (Afifa et al., 2017) explained, the reason for the lack of an increase in INF-Y, which is responsible for the production of killer cells (NK cells), is due to the parasite's infection of the killer cells, which leads to To a decrease in its functions, including the production of INF-Y.

As for TNF- α , a significant increase was observed in the serum of those infected with toxoplasmosis, as this study agreed with (Kadhem et al., 2019) in Diyala and with (Saheb and AL-Issa, 2020) in Mosul, and agreed with (Jameel and AL-Qadhi, 2020; Kanval et al., 2024). in Iraq in a study on cutaneous leishmaniasis, while these results did not agree with (Latifynia and Khansari: 2020, Franca, 2012), as they did not show significant differences for TNF- α for the cutaneous leishmaniasis parasite.

Recent studies have indicated that infection with the parasite inhibits the production of tumor necrosis factor (TNF- α) in order to initiate infection. It is believed to be responsible for producing the acute inflammatory response by activating the antimicrobial activity of infected macrophages by stimulating the production of INF-Y from them and from killer cells. TNF- α works synergistically with INF-Y to develop antibiotic resistance against infections. Therefore, toxoplasmosis plays a crucial role in protective immunity against toxoplasmosis (Sana et al., 2022; Rashid et al., 2023).

In toxoplasmosis, TNF- α appears to be essential for activating macrophages and preventing parasite multiplication, but it can only play this role in synergy with INF- α . This protective role has been observed in mice in both the acute and chronic phase of the disease. In addition, TNF- α , like IL-12, is another product of macrophages, which plays an important role in the early non-specific response during toxoplasmosis, etc. The role of TNF- α in toxoplasmosis is still debated, as some researchers have noted a link between TNF- α and fatal infection in toxoplasmosis in mice, where clinical signs of the disease have been observed in the liver and brain. It may help spread and localize infection within the brain of mice infected with the parasite, and may increase congenital toxoplasmosis during primary infection in humans (Filisetti and Candolfi, 2004; Jam et al., 2014).

Tumor necrosis factor, a potent and multi-active cytokine, causes a wide range of effects on a wide range of cells. It has been shown to affect hormone production, placenta formation, fetal development, and steroid formation. It has an important role in placental differentiation and the birth

process, and at the same time its ability to terminate pregnancy has been proven (Al-Ubaidi and Shareef, 2021).

As for G-CSF, there were no significant differences, and the results of the current study differed with (Jacqueline et al., 2002; Jam et al., 2013) in Lebanon. Both G-CSF and GM-CSF are secreted by human fibroblasts infected with toxoplasmosis, so G-CSF levels rise as an acute phase response in various diseases. Infectious diseases, so it plays an important role in innate immunity.

The concentration of IgG Cysteine, TNF- α , INF-Y, NK cells, IgM, and CSF was measured according to age groups, and it was found that there were highly significant differences of 0.01 for Cysteine in the group of affected women when compared with the control group, and significant differences of 0.05 for TNF- α . α between the group of affected women and compared with the control group, and there were no significant differences for INF-Y, NK cells, and CSF in the group of affected women when compared with the control group as in Table (4).

This study showed that there is a relationship between women infected with toxoplasmosis and age groups. There were significant differences of IgG and IgM at a rate of 0.05 for the age group. This study agreed with a study conducted in Iran that the highest rate of toxoplasmosis was observed in women aged (>20) years. Likewise, a study in India showed an increase in the prevalence of toxoplasmosis in age groups (18-40). It differed with a study in Erbil, which showed that women aged between (21-30) years did not record any statistical differences between those infected with toxoplasmosis (Noor, 2020).

The results showed that there is a negative relationship between age and the immune variable INF-Y at a probability level of 0.05, while there is a positive relationship between age and the immune variable TNF- α at a probability level of 0.05. This explains that the secretion of the immune cytokine INF-Y increased in varying proportions among the age groups that were recorded in the study. It does not agree with Carvalho et al (2015), when it was shown that only elderly people have a decreased response in the level of INF-Y, which leads to a lack of the main cytokine responsible for macrophages. The results of the current study of younger ages also showed a decrease in the level of INF-Y, which is possible. The reason is that NK cells, which are responsible for secreting INF-Y, were not activated.

While the secretion and increase of TNF- α among age groups was consistent with a study conducted on cutaneous leishmaniasis (Oliveira et al., 2011), which showed that the age of the affected person and TNF- α are interrelated and have a relationship to the size of the ulcer. An increase in Cysteine was observed in our study. Current differences according to age groups, where high significant differences were recorded at a probability level of 0.05, and the reason for the increase was an important role in the pathogenicity of the parasite, as it prevents the development of intracellular stages and disrupts the secretory pathway of toxoplasmosis. Likewise, killer cells (NK cells) recorded no significant differences with respect to age group. This is due to the fact that since toxoplasmosis infects cells, it is possible for it to infect killer cells and turn them into parasitic cells, which help in secreting INF-Y. We find in our current study that both killer cells (NK cells) and INF-Y were not active against toxoplasmosis infection.

The current study showed that there were no significant differences in G-CSF for age groups, and this differed from (Torres et al., 2009), where the study was conducted on 65 women aged between (32-39). The results of this study indicated the effect of G-CSF. It is involved in rupture of the follicular wall and may be useful as an adjuvant treatment for LUF.

Table (1): Percentage of women infected with toxoplasmosis

Total	examined samples	No. infected samples	Percentage %
	101	66	65.3

Table (2): Distribution of women infected with toxoplasmosis according to age groups

Age group	Total	examined samples	No.	infected samples	Percentage %
21- 26	61		41		% 67.2
26- 31	39		25		% 64.1
Total	101		66		%65.3

Table (3): Immunological parameters according to toxoplasmosis infection

groups	G-CSF Mean ±	Cysteine Mean ±	TNF-α Mean ±	INF-Y Mean ±	NK cells Mean ±	IgM Mean ±	IgG Mean±
	D. S	D. S	D. S	D. S	D. S	D. S	D. S
infected	±309.7	±9.07	±264	±793	±104.9	0.4 ± 1.872	±42.70
	54.9	2.77	22.5	190	26.6	72	7.011
control	±306.4	±13.48	±403	±829	±125.8	0.1±0.477	± 0.470
	58.9	3.63	25.9	201	30.4	49	0.213
P-value	ns	**	*	ns	ns	**	**
	0.872	0.003	0.048	0.811	0.464	0.0006	0.0004

ns indicates no significant differences

Table (4): Measurement rate of the immune parameters under study according to age groups

Groups	G-CSF	Cysteine	TNF-α	INF-Y	NK cells	IgM	IgG
	Mean ±	Mean ±	Mean ±	Mean ±	Mean ±	Mean ±	Mean±
	D. S	D. S	D. S	D. S	D. S	D. S	D. S
Infected							
21-25	±312.8	±9.04	±243.4	±811.6	±122.9	± 1.747	15.4±52.17
	56.2	2.66	40.5	44.7	27.3	0.437	9
	a	С	b	a	a	a	ab
26-31	±306.0	±10.26	±288.6	± 820.4	±113.1	±2.023	±58.34
	55.2	4.64	38.8	33.2	25.3	0.485	12.84
	Α	bc	b	a	a	a	a
Control							
21-25	±333.1	±17.33	± 419.0	±846	± 134.5	± 0.562	±41.70
	54.3	8.77	31.9	55.2	26.2	0.128	14.01
	a	a	a	a	a	b	bc
26-31	±322.4	±12.87	±384.5	±810	±115.3	0.03 ± 0.345	±37.00
	64.1	4.92	38.8	37.8	28.1	4	9.910
	a	b	a	a	a	b	С
p-value	ns 0.227	**	*	ns	ns	**	*
		0.006	0.055	0.795	0.581	0.01	0.020

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^{*}Indicates that there are differences at the level of 0.05

^{**} Indicates that there are highly significant differences at the level of 0.01

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