ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

IDEATIFICATIONFOR THE LEVEL ANTIBODES (IGG ANDIGM) OF TOXOPLASMA GONDIIIMMNOCOMPROMISEDPATIENTS IN KIRKUK CITY

[1]Abedullkalig,[2] AlwanmhemedQasim,[3] Ibrahim Khalaf
qa.ibrahim69@gmail

Abstract

The current study is aimed to investigate the prevalence of toxoplasmosis in immunosuppressed people by using serological methods (ELISA) and genetic methods polymerase chain reaction (PCR) it also studies their effects on enzymes and liver functions. The study included the collection of 250 blood samples from disease groups (patients with rheumatism who are taking immunosuppressive therapy, patients with Patients with cancerous disease, patients with pulmonary tuberculosis, patients with diabetes and patients with thalassemia). The study also collected samples from healthy people. We used two kits from the ELISA test, the first for the detection of IgM antibodies and the second for the detection of IgG antibodies. The polymerase chain reaction test was also conducted to detect the parasite using two types of primers. Regarding biochemical tests, the level of the total protein, albumin, globulin, cholesterol, triglycerides and glucose was detected. The results of the current study showed that the (percentage of positive cases for the ELISA test used to detect IgM antibodies for patients with rheumatism who are taking immunosuppressive therapy, patients with cancerous tumors, patients with pulmonary tuberculosis, diabetes, and patients with thalassemia were 22%, 18%, 16.6%, 20%, respectively .While the proportion of healthy people was 3.3% and the percentage of positive cases of the ELISA test used to detect IgG antibodies for patients with rheumatism who take immunosuppressive therapy, patients with cancerous tumors, patients with tuberculosis, diabetes, and patients with thalassemia were 26%, 22%, 20%, 30%, 26.6%, respectively.While the proportion in healthy subjects was 10% and positive cases for both types of antibodies were recorded for the groups, patients with cancerous tumors, patients with pulmonary tuberculosis, diabetes patients, and patients with thalassemia were 10%, 16%, 7.5%, 13.3%, 13.3%, respectively .

Introduction

Toxoplasmosis caused by the parasite Toxoplasma gondii is a disease of global spread in most population groups due to the ability of this parasite to cause infection and reproduction in almost all the nucleated cells of milk and birds. There are three infective stages of T. gondii, which are the tachyzoite, which divide strongly within all cells of the final and intermediate host except for the intestinal epithelial cells of the second host, and the bradyzoite stage, which reproduces slowly within the cyst of tissue formed inside the cells of the host body and which varies in size and shape Depending on the age and location of the infection, the oocyst is developed, which is excreted with the feces of infected cats, to form the infected spores later. Transmission of infection to humans occurs mainly through eating foods or drinks contaminated with infected sacs of the inoculum, or eating undercooked meat containing live tissue sacs, as Kasper (1998) showed that eating a single mature inoculum sac is sufficient to cause infection in humans. Blood is an important source of transmission of infection in the acute phase, and the same is true in transfusions of organs and tissues, which may be infectious to healthy people who are given immunosuppressive drugs, which play an important role in the occurrence of active infection (Slavin&Mayers., 1994). Acute toxoplasmosis is characterized by its spread And the rapid intracellular growth of the rapid alveoli from the intestine to the various organs of the host (Channon et al., 2000) causes necrosis and the formation of lesions resulting in an inflammatory reaction that leads to the emergence of symptoms that range from fever, headaches and anemia to complications that lead to Destruction of cells in the lungs, liver, heart, brain, eyes, and sometimes the involvement of the central nervous system (Simpson., 2002). However, in immunocompetent individuals, the infection becomes chronic, accompanied by a halt in the multiplication of rapid follicles and tissue cysts containing slow follicles that remain within the tissues for several years .without causing any clinical effects. Toxoplasmosis is a common disease in the world, especially in hot, humid regions .

Materials and method Samples

During the study, 200 samples were collected from inpatients and arrivals to Kirkuk hospitals, and 50 samples were collected from healthy people, according to

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

Table (3-1): The number of samples distributed according to the groups included in the study

Categories	Number of samples
Rheumatoid patients taking immunosuppressive drugs	50
patients with carcinomas.	50
pulmonary tuberculosis patients .	40
Diabetics	30
. Patients with Thalassemia	30
Healthy	50
Total	250

Workingmethods

Collection of blood samples: 10 ml of each person were collected by 5 ml in anticoagulant-free tubes (for the -1-2-3 purpose of separating serum for ELISA and biochemical tests) and 5 ml for the purpose of post-testing, the anticoagulant-free tubes were left at an angle at room temperature (25 C) for 30 minutes, then separated by centrifuge at 1000 rpm for 15 minutes. The serum was separated and withdrawn with a Pasteur pipette and kept in sterile tubes. The tubes were kept in Deep Freeze at -80 °C until immunological and biochemical tests were performed

ELISA test

ELISA test for the detection of IgM antibodies against Toxoplasma

Basis of the test: The test depends on the use of coating the pits in the plate with Toxoplasma antigens. When adding the patient's serum containing Toxoplasma IgM antibodies, the antibodies bind to the Toxoplasm antigens installed on the walls of the pits. The pits are washed to remove the unbound antibodies and then the antibody is added (Anti T.gondii IgM which is associated with the peroxidase enzyme, then the substrate is added, which interacts with the enzyme, leading to a color change that is in intensity proportional to the concentration of antibodies in the serum (Naot& Remington, 1980)

1-Serum samples, negative and positive control, and inhibiting solution were diluted in a ratio of 1:40 (5 µl of sample + .(200 µl of dilution solution

2µl - of the diluted samples were transferred to the etching of an ELISA dish100

3-Use the shaker to move the plate and remove air bubbles

4-.Incubate the dish in the incubator at 37°C for 30 minutes

5- Remove the non-adherent antibodies by pouring and washing them four times

.6- of coupling solution was added to all pits and the plate was left for 10 seconds100µl

7-.The dishes were incubated at 37°C for 30 minutes

8-Remove the non-adherent coupling solution by pouring the dish and washing it four times

.9-of the substrate solution was added and the plate was gently stirred for 10 seconds

10.The dish was incubated at 37°C for 15 minutes

.1l of suspension solution was added to the reaction and the plate was stirred slowlµl100 y for 30 seconds

12-A color change from blue to completely yellow in the case of a positive result, and to a transparent color in the case of a negative result

13-The values at a wavelength of 450 nm were read in 15 minutes by the reader connected to the device Microwell .Reader

-:The function is calculated based on the inhibitory serum and my agencies

.-If the Toxo-IgM function is less than or equal to 0.90 IU/ml, the result is negative

The Toxo-IgM function ranges between 0.91 - 0.99 IU/ml, the result is non-equivocal and the model must be re- .examined

If the Toxo-IgM function is greater than or equal to 1.00 IU/ml, the result is positive

Results and discussion

The results of the ELISA test

Results of the ELISA test for the detection of Toxoplasma gondii antibodies

In the current study, two ELISA test kits were used, the first for the detection of IgM antibodies and the other for the detection of IgG antibodies. The result was considered positive if one or both types were detected. Table (4-1) shows the results of the ELISA test. It is noted that the highest percentage recorded In patients with diabetes and thalassemia,

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

it reached 60%, while the rate of infection among patients with rheumatism who were taking immunosuppressive treatments was 58%, and the percentage in patients with cancerous tumors was 56% and in patients with pulmonary (tuberculosis 43%, while the ELISA test gave positive results amounting to 13.3% In healthy subjects (control group

Table (4-1): The results of the ELISA test are distributed according to the target groups in the study

Categories	Positive ELISA Test		Negative ELISA Test	
	The number	The percentage	The number	The percentage
Patients with rheumatism taking (immunosuppressive therapies (50)	29	%58	21	%42
Patients with carcinomas (50)	28	%56.0	22	%44
(Patients with pulmonary tuberculosis(40	17	%43	23	%57
Diabetics(30)	18	%60	12	%40
Thalassemia patients (30)	18	%60	12	%40
Healthy(50)	4	%13.3	46	%86.6

The results of the ELISA test are distributed according to the type of antibodies and the target group of patients in the study

Table (4-2) shows the results of the ELISA test, distributed according to the type of antibodies and the target group. In general, it is noted that the appearance of IgG antibodies was more than the appearance of IgM antibodies and for each target group, and with regard to IgM antibodies, it is noted that the highest appearance of them was when Patients with thalassemia, at a rate of 22%, followed by patients with rheumatism who take immunosuppressive treatments, at rates of 20%, and the lowest appearance of antibodies was in patients with pulmonary tuberculosis, at rates of 15%, while the percentage of antibodies appearing in healthy people was 3.3%. As for IgG antibodies, it is noted that The highest back was in diabetic patients with a percentage of 30%, and the lowest appearance was in those with .tuberculosis by 20%. As for healthy people, they showed IgG antibodies by 10%

Table (4-2): The results of the ELISA test are distributed according to the type of antibodies, the target groups in the study

Categories	Type Antibodies			
	IgM	IgG	IgM IgG	
Patients with rheumatism taking immunosuppressive therapies (50)	11 %22	13 %26	5 %10	21 %42
Patients with carcinomas (50)	9 %18	11 8 %22	22 %16	22 %44
Patients with pulmonary tuberculosis(40)	%615	8 %20	3 %7.5	23 %57
Diabetics (30)	5 %16.6	9 %30	4 %13.3	12 %40
Thalassemia patients (30)	%620 %26.6	8 %26	%413.3	%1240

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

(Healthy(50	%13.3	%310	%00	%4686.6

These studies are characterized as being among the few studies in Iraq that have adopted modern technologies (genetic testing and ELISA testing) to shed light on the relationship between immunosuppressive diseases and toxoplasmosis. In recent years, toxoplasmosis has gained great importance as an opportunistic pathogen, especially in immunocompromised patients. Malignant tumors such as leukemias, lymphomas, AIDS patients, organ transplants, and immunosuppression are known to be predisposing factors for toxoplasmosis (James, 2004; Koltas et.al, 1989; Breecher., .(1999

The results of the current study showed that the percentage of infection (IgM and /or IgG) in patients with cancerous tumors and patients with thalassemia was 56% and 60%, respectively. This percentage is lower than the percentage .recorded before (1991, Khalil et al.), which was 36%

The appearance of higher rates of infection in people taking anti-cancer agents (cytotoxic agent like doxorubicin, daunorubicin, methotrexate, cytosine, and mercaptopurine. DNA, therefore, causes cell death (Laurence et.al, 1997), and these treatments cause loss of appetite, diarrhea, vomiting, inflammation of the intestinal lining, and a defect in food absorption, and thus cause malnutrition, low levels of proteins, and weak immunity (Salmon and Sartorelli, 2001) (record)) Al Khaffaf.,2000 recorded an infection rate of 69%, while (Tabbara et. Al., 1999) recorded an infection rate of 36%, and perhaps the reason for this high percentage compared to the percentage recorded in the study is due to the difference in the type of test. Infected people compared to healthy people due to the nature of chemotherapy and radiation, which suppresses the work of the immune system (Pearl et al., 1995). It was found by Asci and his colleagues (1997) that the percentage of emergence of antibodies was (55.3%), and the researcher found Al-Samani in the year (20) 00) in a study on the prevalence of toxoplasmosis in pregnant and aborted women, which amounted to (43.6%), as well as the researcher Abdullah in the year (2001), which gave an infection rate of (56.6%), while the researcher .explained the pumice in a serological study of toxoplasmosis

In (2001) the incidence of toxoplasmosis was (69.2%), the results obtained by Al-Dulaimi (2002) (2005. Al-Wattari) and Al-Doski., 2000) and in Erbil, where the rates of infection were 48.7%, 36.7%,) .in Mosul (Othman. 2004) and in Kirkuk 36.67%, 49.85%, while the study (Al-Ghurairi, 2006) recorded infection rates in Diyala, Baghdad and Anbar amounting to Abdul-Mohymen et al., 2009, 2011). The reason for the difference between the results we obtained) %32 %29 ,%33.3 with the results of the researchers above is due to the difference in the category of patients targeted in the study, the type of test and even the type of antibodies. Target The results of our study differed from the results obtained by (Ramadan et.al 2000), which amounted to 4.8%, and the reason for the difference is the type of test adopted and the target group, where the researcher relied on the stacking test, which is less sensitive than ELISA, and his study was a non-target survey. In the current study, two types of antibodies were measured, IgG and IgM using the ELISA test, and the results were evaluated depending on the appearance of one or both types of antibodies. The appearance of IgM antibodies indicates acute and active infection, and the appearance of IgG antibodies indicates chronic infection, while .The appearance of both types of IgG and IgM antibodies indicates that the infection is old and still effective

The rise of IgM antibodies begins a week after infection and is an indicator of the emergence of acute infection and reaches its highest level in the second week and then begins to decline gradually and may remain at low levels for a period of more than 6 months, while IgG antibodies begin to appear after the third week and gradually rise and then decline Low levels can be kept for longevity (Duby., 1986). The study of Bertozzi et al., 1999) indicated that the level of IgM antibodies can persist for four years. The occurrence of fluctuation in the appearance of two types of antibodies is due to the fact that the parasite remains for a long time in the body of the body until it reaches a state of equilibrium, but the occurrence of any decrease or decline in the immune state of the body for any reason will lead to the activation .of tissue cysts as a whole

Conclusions

.1-A high prevalence of toxoplasmosis in immunosuppressed patients compared to healthy individuals

2- The two disease groups: Thalassemia patients and diabetic patients are the most vulnerable groups to infection

.3-In general, the high level of IgG antibodies was more than that of IgM antibodies for all study groups

REFERENCS

Slavin, M. A. and J. D. Mayers. 1994. Toxoplasma gondii infection in marrow transplantation recipients: a 20 year .experience. Bone marrow transplant, 13: 549-557

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

Simpson, 2002.Toxoplasma gondii-Life Cycle, Morphology, Pathogenesis, Attachment to and Entry into the Host .(cell.(Internet
.Remington, J. S.; R. Mcleod; P. Thulliez and G. Desmonts
In: Remington, J. S. (Ed.). Infectious diseases of the fetus .2001
-and newborn infant. W. B. Saunders Co. Philadelphia. pp. 205
.346
.Romand, S.; Chosson, M.; Franck, J.; Wallon, M. and Kieffer, F
Usefulness of quantitative polymerase chain reaction in .(2004)
amniotic fluid as early prognostic marker of fetal infection with
.Toxoplasma gondii. Am. J. Obstet. Gynecol. 190:797–802
,Buxton, D.; Brebner, J.; Wright, S.; Maley, S.W.; Thomson
K.M. and Millard, K. (2016).Decoquinate and the control of
-experimental ovine toxoplasmosis. Vet. Res., 128(18):434
.436
(, Khalil et al(Tabbara et. Al., 19991991),James, 2004; Koltas et.al

Salmon and Sartorelli, 2001Duby., 1986)
Ramadan et.al 2000)