J. Cardiovasc Disease Res., 2020; 11(4): 141 – 145
A Multifaceted Peer Reviewed Journal in the field of Cardiology
www.jcdronline.org | www.journalonweb.com/jcdr
P – ISSN: 0975 – 3583 Online: 0976 – 2833

Diagnosis of *Toxoplasma gondii* by Using ANA Test with Study of the Effect of the Parasite on Ceruloplasmin Enzyme

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ABSTRACT

This study included detection of toxoplasmosis in pregnant and aborted women, reviews of medical centers and private external laboratories in the city of Samarra, and 100 samples were obtained for the period between November 2018 to February 2019 and their ages ranged from (15 - 45) years and the patient's form that included The following information: age, housing, number of children, number of miscarriages, presence of animals, especially cats. Blood was examined to detect IgG, IgM antibody by cassette method, and the ratio of antibody to the ANA nucleus and ceruloplasmin and its relationship to infection was measured. The current study found that the incidence of acute infection with the presence of IgM antibody was 60% and chronic infection in the presence of IgG antibody reached 40%. The result of the examination of ANA antibodies showed a significant increase in the level (P <0.05) compared to the control group, whereas the seroplasmin enzyme increased significantly with P <0.05) compared to the control group.

Keywords: *Toxoplasma gondii*, ANA test, ceruloplasmin enzyme.

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Submitted: 26-09-2020
Revision: 22-10-2020
Accepted Date: 13-11-2020

DOI: 10.31838/jcdr.2020.11.04.24

INTRODUCTION

Toxoplasmosis is a common disease between humans and animals, and it is one of the diseases currently widespread in most parts of the world, including the Arab world, including Iraq (1), the infection causes this parasite called the *Toxoplasma gondii*, which is characterized by its ability to infect a huge number of intermediate hosts represented by All types of mammals and a number of birds and reptiles (2), where the disease is present in two forms, depending on the method of infection, the first form is toxoplasmosis that occurs as a result of the transmission of the parasite from the mother to her fetus through the placenta, the second form results from the acquisition of infection with contaminated water and food. This parasite is characterized by having several phases responsible for transferring the infection to the intermediate hosts. The process of transmission of the infection to the human being is by eating the uncooked meat that contains the tissue bags of the parasite, as well as eating vegetables and fruits contaminated with the egg sacs, as well as Transmission of the infection through the placenta from the mother to the fetus (2). The severity of this infection in all types of hosts ranges from mild, acute or chronic, It is without symptoms in the competent host immunologically, or is accompanied by influenza-like symptoms (3). The host's immune system can stop the parasite's duplication and tissue cyst formation in most body tissues, which are mainly concentrated in Central Nervous system, Skeletal Muscles, Cardiac Muscles and without any symptoms in most cases or accompanied by mild symptoms, and the severity of the disease may worsen in Immunocompromised Or people who take drugs to treat tumors, especially those with AIDS (4).

Toxoplasmosis is a widespread disease, about a third of the world's population may have antibodies against it, which means they may become infected at some point in their lives (5), Cats are the most common source of this disease, as they are known to be the main transmitters of infection, as well as birds, rodents and undercooked meat. The danger of the disease lies when pregnant women are exposed to the disease, as infection may be transmitted to the fetus by the placenta leading to miscarriage, death in the womb, or causing birth defects (6). Objectives of the study are detection of specific antibodies against IgG, IgM toxoplasmosis in pregnant women using Cassette rapid test strip, detection of ANA antibody by ELISA and its relationship to infection, and measuring the ratio of the ceruloplasmin enzyme in the liver and its effect on the infection with the parasite.

MATERIAL AND METHODS

Sample collection

This study was conducted for the period of November 2018 to February 2019 and included the collection of (100) samples for pregnant women who reviewed the primary health care center in the teachers 'district and reviews for some private laboratories and their ages ranged from (15 - 45) years and the patient's form was filled in and included the following information: Age, housing, the number of children, the number of miscarriages, the presence of animals, especially cats. Blood samples were obtained intravenously by 10 ml from each woman, using a syringe. Then it was put in special laboratory tubes in the centrifuge for 15 minutes at a speed of 3000 rpm, and divided the serum resulting from the separation process and using a micropipette to the following:

1-Putting 1 ml of the resulting serum into sterile and labeled 5 ml plastic tubes and then placed in the freezer at a temperature of -20 ° C in order to preserve in case of re-examination or the need for more serum.

2- Place the remainder of the serum in a Plain tube free of any anticoagulant, for the purpose of conducting IgG, IgM antibody detection of the toxoplasma parasite and conducting other checks. The samples that contained the RBC degradation were neglected, for the sake of accuracy in

the results each model was numbered and the name and date written.

Detection of specific antibody

A quantity of the serum was withdrawn by means of a micropipette of 20, and placed in the place for the sample to be placed in the test strip designated for the examination of IgM or IgG specific antibodies. Then two drops of Diluent Solution were added (Figure 1). Then I left 15 minutes until the result appears in the test strip. When the (red) indicator appears on the (C) Control line, this indicates that the sample is negative. When the indicator appears on (G), this indicates that the sample is positive for IgG specific antibodies. When the indicator appears on (M), this indicates that the sample is positive for IgM specific antibodies.

Figure 1: Method to detect the presence of specific antibodies (Leaflet)

Determination of Antinuclear antibody

The ANA concentration was estimated by following the steps provided with its ready-made analysis kit and according to the manufacturer's instructions for ELISA technology (7).

Method of testing ceruloplasmin

The method was conducted by depended Kit LSBIO according to manufacturer.

RESULTS AND DISCUSSION

The results of immunological examinations

Acute and time infection: The results of the current study showed that the proportion of infection distribution according to antibodies is represented by high IgM and acute infection by (60%), while IgG antibody has reached (40%) and the highest infection rate against IgG has reached (100%) at age (35) -40) As for IgM, it reached the highest rise in age (30-35) by (23%) and agrees with (8) where the percentage of latent infection with the emergence of antibody IgG increased by (33%) and acute infection represented by IgM by (46%) and with (9) Where IgG reached 10.5% and IgM increased by 34.1%, while it did not agree with (10) in the city of Baghdad, it showed the percentage of latent and acute infection (14%) and (4%), respectively, and it did not agree with (11) in Al-Diwaniyah city. The ratio of latent and immediate infection D were (33.6%) and (17.6%) respectively, and with (12) 30% 70% Igm and IgG respectively and (13) Igm and IgG at 14.2% and 9.1% respectively, Sera positivity was explained according to what the researcher reported (14), since it was stated that the positive of the IgM antibody test alone indicates an acute infection early and IgG should be performed within several weeks and the positive test then proves the patient's injury with Toxoplasma. As for the recent infection it is referred to the latent infection when The IgM result is negative and the IgG result is positive and accordingly we find a high percentage of the injury results are recent infection.

Table 1: Incidence rate according to type IgG or IgM

Age group	Number of samples tested	Number of infected samples	IgG	%	IgM	%
15-20	19	/	/	/	/	/
20-25	28	8	3	10.7	5	17.8
25-30	25	7	3	12	4	16
30-35	13	4	1	7.6	3	23
35-40	15	1	1	/	/	100
Total	100	20	8*	40	12*	60

* Indicates significant differences at probability level (P <0.05).

ANA test results

The antibody test for the nucleus is considered one of the tests that are used to detect autoimmune diseases. These diseases include diabetes, rheumatoid arthritis, lupus, etc. Some studies have found that infection with *T. gondii* plays an important role in causing many autoimmune diseases. A study (15) confirmed the occurrence of a high rate of ANA in people with toxoplasma and this is consistent with our results, where our current study found an increase in the level of ANA among people with toxoplasma as in Table (2), as the concentration in the group of infected women reached (0.707857 ± .2160360MUI / ml) compared with a group Control as it reached infectious Where concentration (0.243387 ± 1.42026)) MUI / ml.

Table 2: Mean ± standard error of ANA concentration (pg / ml) for affected women compared Control group

The group	NO.	ANA Average ± standard error
Control	25	0.243387 ± 1. 42026
Infection	25	0.707857 ± .2160360 *

* Indicates significant differences at probability level (P <0.05).

While (20) stated that patients with autoimmune diseases are at risk of developing toxoplasmosis, the reason for this may be due to the use of anti-immunomodulatory drugs such as MTX that have a major role in the immune response against toxoplasmosis infection since the role of this factor begins when Entering Tacyzoite where macrophages stimulate the production of 12-IL-12, which in turn stimulates killer cells as well as T-cells to produce IFN-γ which works in conjunction with TNF-α. Tumor Necrotic Factor, killing Tacyzoite, and the interconnection between these two factors secretes Free production radicals and (NO) which have the ability to kill the parasite as that And Nitric oxide is of great importance and decisive resistance to the parasite through its important role in the production of HSP70 protein (21), This protein is produced in mammalian cell types, including smooth muscle cells of the vessels and hepatocytes, and that the HSP70 has an important defensive role to protect cells from toxic effects as it contributes to controlling toxoplasmosis parasites and gives the host more immunity, by preventing the parasite replication inside or in the macrophages. (22 and 23) Since methotrexate therapy affects the production of neoplastic necrosis factor alpha, it does not get an immune response against the parasite and consequently the occurrence of the infection corresponds to (24 and 25), as women with latent parasites had significantly higher levels in the level of blood glucose.

While it did not agree with (27), where he did not find a relationship between toxoplasmosis and autoimmune diseases (28), as well as with (29), who stated that infection with toxoplasma has no role in improving autoimmune diseases, where he indicated when the parasite is infected, the production of IL-10 gets interruption. Which has a role in the decrease of IL-17 to prevent infection with immune diseases, where IL-10 Anti - inflammatory, (30) is secreted by B Cell, Th2 and Tregs - CD8 (31), as well as produced from cells that have a role in inherent immunity such as Dentric Cell, Natural Killer Cell (NK), Neutrophils, eosinophils, Macrophage, and Mast Cell. Therefore, IL-10 is responsible for the important regulatory role it plays in regulating and defining the immune response (32) It is also called Cytokine synthesis inhibitory factor (CSIF) and is from the cytokines family that includes both (IL - 19, 20, 22, 24, 26). Where 10 (IL-10) has positive and negative effects, because its production from different immune cells is considered Pleiotropic, and one of its positive effects is that it stimulates phagocytic cells on the process of phagocytosis and urges the production of TNF-∝, IFN-γ from natural killer cells (NK) It works to stimulate activated B-cells to replicate and differentiate into antibody-secreting cells. It also works to determine the immune response and prevents host cell breakdown and tissue damage due to inflammation by resurrection by maintaining the epithelial barrier of the intestine (33).

Ceruloplasmin test results

T.gondii parasite remains a global public health problem (34), despite significant effort and significant progress, toxoplasmosis remains a major threat to global health, and pathophysiology remains not fully understood and especially the effect of infection on liver metabolism in the host. In this study, the effect of the parasite on the ceruloplasmin enzyme, which is one of the important enzymes present in the blood that transports copper atoms to the liver cells, has been investigated, and its function is related to excess storage of copper in the liver, brain and other organs. Some scientists believe that low production of ceruloplasmin may be due to a defect in the liver's ability to break down copper (35)(36).

Some studies have indicated that the liver is another organ targeted for the reproduction of *T. gondii*, as evidenced by many liver diseases, such as hepatomegaly, hepatitis, granuloma, necrosis, biliary jaundice, and cirrhosis (37) associated with *T. gondii* infection. Although parasitic and host factors are generally known to contribute to the development and development of toxoplasmosis, the interaction between *T. gondii* and the host liver is still not understood. Given the fact that the liver is an essential organ that can perform a wide range of critical processes, such as detoxification from microorganisms and bile production that facilitate digestion. Functional change in the liver may lead to severe illness, such as cancer, malnutrition, hepatitis, jaundice, cirrhosis and harmful drug interactions (38). Functional change in the liver leads to severe disease, such as cancer, malnutrition, hepatitis, jaundice, cirrhosis and harmful drug interactions (39). Table (3) showed an increase in the level of the enzyme, as it reached a concentration in the infected group (32.944 ± 11.3875 (MU / ml) compared to the control group, as the concentration rate in it reached 37.38333 ± 8.273149) MU / ml.

Table 3: Modifier ± standard error of ceruloplasmin enzyme concentration (mu / ml) for affected women compared to control group

The group	NO.	ANA Average ± standard error
Control	25	37.38333 ± 8.273149
Infection	25	32.944 ± 11.3875 *

* Indicates significant differences at probability level (P <0.05).

These results were consistent with (39,40), where the enzyme rate increased in toxoplasmosis women, and Ceruloplasmin was used widely as a measure of acute phase reaction and as an antioxidant in inflammatory cases (41), and in study (42) the copper level was measured as a method It is preferred to detect infection during the first three months of pregnancy as the level of this enzyme in the blood reflects the occurrence of liver damage or muscle damage, as our study agreed with (43) in Turkey that was conducted on dogs with the parasite and had recorded a higher celluloplasmin concentration in infected dogs *T. gondii* Compared with the control group (P <0.01), it also indicated that celluloplasmin is portion developed acute responds moderately inflammatory and tissue damage, and supports these studies also the idea that the high concentration of serum Ceruloplasmin can occur during both phases of recovery from inflammation (44).

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Cite this article: Ohood Mozahim Shakir. Diagnosis of Toxoplasma gondii by Using ANA Test with Study of the Effect of the Parasite on Ceruloplasmin Enzyme. J. Cardiovascular Disease Res., 2020; 11 (4): 141 – 145