Short Communication

Serological and Molecular Diagnosis of Toxoplasmosis in Some Cancer Patients

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Abstract

Background: *Toxoplasma gondii*, a parasite affecting both humans and animals globally, is an obligate intracellular protozoan. Immunosuppressed patients have a high prevalence of toxoplasma infection. For some immunocompromised individuals, such as those with autoimmune diseases, 90% of toxoplasmosis cases in immunocompromised people such as cancer patients are typically asymptomatic, with the remaining 10% displaying flu-like symptoms, ophthalmic illness, or cervical lymphadenopathy. Toxoplasmosis is severe and could be fatal. **Objective:** The purpose of this study was to identify Immunoglobulin g (IgG) and Immunoglobulin m (IgM) antibodies and perform a molecular assay against toxoplasma in malignant patients to promptly refer the patients to a doctor for treatment. **Materials and Methods:** This case-control study was done on 7–75 years old cancer patients and was carried out on 70 cancer patients and 20 healthy controls obtained from Kirkuk Oncology and Hematology Center. Patients' information was recorded before sampling. Blood samples were examined for various serological tests (latex agglutination test [LAT], rapid immunochromatographic test [RITC], and enzyme-linked immunosorbent assay [ELISA]) and molecular analysis (polymerase chain reaction). **Results:** The findings indicate that an ELISA test revealed some subjects had toxoplasmosis infections in cancer patients—7 (10%) IgG+/IgM+, 11 (15.71%) IgG+/IgM−, 4 (5.71%) IgG−/IgM+, and 48 (68.58%) IgG−/IgM−. The seropositive rate of *T. gondii* in cancer patients, according to LAT, was 33 (47.1%), in RITC it was 34 (48.6%), and in ELISA test it was 22 (31.4%). Molecular result of the PCR showed 2 (4%) positives and 14 (28%) negatives. **Conclusions:** Before, during, and after chemotherapy, patients must be tested for toxoplasmosis based on the frequency of positive cases in these patients.

Keywords: Cancer patients, diagnosis, molecular, serological

Introduction

An obligatory intracellular protozoan parasite, *Toxoplasma gondii*, affects both humans and animals worldwide. The disease is transmitted to humans in two ways: either by consuming raw, uncooked meat from infected animals or by ingesting *T. gondii* oocysts released into the environment by cat feces.[1]

Recent research, however, has backed up the importance of infection reactivation in immunocompromised patients and the clinical impact of *T. gondii*.[2] In immunocompetent individuals, 90% of toxoplasmosis cases go undiagnosed, with the remaining 10% presenting with signs such as flu-like symptoms and ophthalmic illness.[3] Sometimes toxoplasmosis can be severe and could be fatal.[4]

Toxoplasma gondii has been implicated in earlier investigations as a significant opportunistic pathogen in immunocompromised individuals. Recent research performed a global meta-analysis and assessed the prevalence of *T. gondii* infection in immunocompromised individuals by studying electronic databases for *T. gondii* infection in human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) patients, cancer patients, pregnant women, and transplant patients in a total of 72 eligible studies.[5,6]

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Toxoplasma gondii enters a latent or dormant phase in healthy humans, offering lifetime immunity from this parasite; however, the growth of bradyzoites may cause damage to the tissues, resulting in lesions.[7]

However, suppose the person's immunity is compromised due to a disease (such as HIV and cancer) or by taking specific medications like corticosteroids, chemotherapy, or radiotherapy, *T. gondii*, in that case, may reactivate and transform into a tachyzoite stage, where they can start an acute infection by attacking the surrounding tissue.[4] Because of the nature of their immunosuppressive therapy, cancer patients are assumed to be immunocompromised, and as a result, it is anticipated that they will have a higher chance of getting toxoplasmosis (new infection or reactivation from past infection) compared to controls.

The current study sought to examine cancer patients' seropositivity for *T. gondii* antibodies (Immunoglobulin g [IgG] and Immunoglobulin m [IgM]) and for the detection of gene B1 for *T. gondii*.

Materials and Methods

Study of the design and patient recruitment

A cross-sectional study was carried out between November 2022 and April 2023 at the Kirkuk Oncology & Hematology Center to investigate the presence of anti-*T. gondii* in cancer patients and their levels were compared to those of healthy individuals. Before blood was taken, each participant individually completed a consent form. In the study, 70 cancer patients and 20 healthy individuals served as controls. A structured questionnaire was used to collect demographic information from both patients and controls.

Sampling procedures

A licensed registered nurse collected blood from the participants at the center. A single syringe was given to each participant to draw a total of 5 cc of blood. After that, the blood was separated into two tubes, one for serological assay and the other without ethylene diamine tetraacetic acid (EDTA). The sera were divided and both were kept at −20°C until examination.

Laboratory assays

Serological evaluation

A. Latex agglutination test (LAT) for toxoplasma. According to the plasmatic laboratory product, add 50 µL of the serum sample to the center of slide section one after letting the latex reagent and serum sample reach room temperature, then gently shake the latex reagent to resuspend precipitated latex particles containing 0.1% sodium azide preservative, and then add roughly one drop of latex reagent to the serum sample. Use wooden stirrers to mix both drops and cover the entire surface of the slide division. After waiting for 5 min after mixing, check the test findings with your eyes or lens. If there is definite agglutination, the outcome is positive; otherwise, if there is a homogeneous appearance (agglutination did not form), the outcome is negative.[8]

B. Toxo IgG/IgM combo rapid chromatographic immune assay test. According to CTK, Biotech, the sample was thawed if frozen or chilled, then it was mixed before running the experiment. Open the pouch and take the materials out when you are ready to test it. Place the test instrument on a spotless, level surface. Label the gadget with the specimen's ID number. Fill the capillary tube with the specimen up to the specimen line. Two drops of sample diluent were then added to the sample, which had a volume of approximately 10 µL. Set the timer, read the outcome after 10 min, and after 1 min, promising results may be apparent. Negative results must be verified for 15 min.[9]

C. Enzyme-linked immunosorbent assay (ELISA). According to the DRG, detect Toxo IgG and IgM using enzyme-linked immunosorbent assay (ELISA) and place 100 µL of diluted sera, standards, and controls in the relevant wells. Dispense 100 µL of sample diluents in the well A1 location for the reagent blank. Cover the wells with the foils included in the kit. Incubate for 60 min at 37°C after the incubation period, drain the liquid from each well, and rinse the microtiter wells five times with diluted wash solution (300 µL per well). To remove any lingering drops, strike the wells on the absorbent paper strongly. Add 100 µL of an enzyme conjugate (Horseradish peroxidase) to each well. Incubate for 30 min at room temperature. Avoid direct sunlight. Remove all the wells with enzyme conjugates. Rinse the microtiter wells 5 times with diluted wash solution (300 µL per well) to get rid of any leftover drips, strike the wells firmly on absorbent paper, then add 100 µL of the substrate solution into each well. Incubate at room temperature for 15 min. Add 100 µL of stop solution to each well. Its imperative to ensure that the blue color completely transforms into yellow. Within 30 min of adding the stop solution, the optical density at 450 nm should be measured using a microtiter plate reader.[10]

Molecular assay

Nuclear DNA (n DNA) and mitochondrial DNA (m DNA) extraction

Prepare the required number of 1.5 mL disposable polypropylene microcentrifuge tubes per the instructions on the Sacace kit. Include one tube for the extraction's negative control and one for the positive control. Add 10 µL of internal control and 300 µL of lysis solution. Add 100 µL of sample using pipette tips with aerosol barriers. Vortex the tubes. Incubate for 5 min at 65 °C. Centrifuge for 7–10 s. Add 400 µL of prec solution. Add 500 µL of wash solution 1 to each tube using a micropipette with an aerosol barrier tip. Vortex and centrifuge all tubes at 13,000 rpm for 60 s. Use a micropipette with an aerosol barrier tip to add 200 µL.

Real-Time PCR for detection of Toxoplasma gene B1

Prepare the required number of tubes with a fresh, sterile tube reaction mix for amplification of DNA from clinical and control samples, as directed by the Sacace kit. Mix 10*N 1 of *T. gondii* PCR-mix-1-FRT, 5.0*N of PCR-mix-2-FRT, and 0.5*N of polymerase (Taq f) for each sample. Add 15 mL of the reaction mixture and 10 mL of the extracted DNA sample to the appropriate tube, vortex, and centrifuge for 2–3 s. Pipette the mixture, do the control amplification test, and add 10 µL of DNA buffer to the negative control amplification (NCA)-labeled tube and 10 µL of positive control complex to the PCA-labeled tube before placing the tubes in the heat cycler.[10]

Statistical analysis

Descriptive statistics was done using percentages and numbers with Yes and No. A specific chi-square test was used to detect the relation between the studied variables and a *P* value at 0.05 was considered significant.

Ethical approval

Certain ethical approval was taken from Kirkuk's health directorate after completing the final seminar in the department.

Results

Cancer patient's questionnaire

Cancer patients aged ranged from 8 to 75 years. In addition, each patient was diagnosed with a different type of cancer-related toxoplasmosis.

LAT, RITC, and ELISA, and molecular diagnosis of *T. gondii* in autistic children

Out of the 90 serum samples that were serologically tested for toxoplasmosis, 33/70 samples (47.1%) were positive for LAT in cancer patients, with the highest rate being negative 37 (52.9%) in contrast to the control groups, which had 18 (90%). Rapid immuno-chromatographic test (RITC) had 34 positives (48.57%), with the highest rate of negatives being 36 (51.4%), in contrast to the control group, which had 15 (75%) negatives. ELISA showed 22 positives (31.43%), with the highest rate of negativity being 48 (68.6%), in contrast to the control group, which had 18 (90%) negatives. PCR had 2 (4%) positives and 14 (28%) negatives, as shown in Table 1.

Table 2 shows the seroprevalence of *T. gondii* antibodies (Toxo IgG and Toxo IgM) in total of 70 cancer patients and 20 healthy controls by using ELISA techniques.

Table 3 represents positive and negative results of PCR in 16 cancer patients and 3 healthy controls for detection of gene B1 for *T. gondii* according to number, age, and gender.

Discussion

By identifying IgG/IgM antibodies, the current findings provide information on cancer patients. This is in line with earlier research that showed infections with *T. gondii* in immunocompromised patients by LAT and RITC, followed by immunoglobin IgG/IgM assay, and by using PCR to detect gene B1 for *T. gondii*, as shown in Table 1. ELISA analysis showed a nonsignificant *P* value of 0.908, as shown in Table 2.

Table 1: Number of cancer patients and control groups according to the different serological and molecular laboratory techniques

Laboratory techniques		Seroprevalence of toxoplasmosis for cancer patients	Control groups	Chi-square and *P* value
Serological test				
LAT	Positive	33 (47.1%)	2 (10%)	**
	Negative	37 (52.9%)	18 (90%)	**Chi-square = 9.030**
	Total	**70 (100%)**	**20 (100%)**	***P* value = 0.003**
RITC	Positive	34 (48.6%)	5 (25%)	*
	Negative	36 (51.4%)	15 (75%)	**Chi-square = 3.520**
	Total	**70 (100%)**	**20 (100%)**	***P* value = 0.051**
ELISA	Positive	22 (31.4%)	2 (10%)	*
	Negative	48 (68.6%)	18 (90%)	**Chi-square = 3.653**
	Total	**70 (100%)**	**20 (100%)**	***P* value = 0.052**
Molecular test				
PCR	Positive	2 (4%)	0	NS
	Negative	14 (28%)	3 (6%)	Chi-square= 0.419
	Total	**16 (32%)**	**3 (6%)**	*P* value = 0.815

RITC: rapid immuno-chromatographic test

*Significant

**Highly significant

Table 2: Summary of *T. gondii* antibodies seroprevalence in patients and controls by using ELISA technique

Seroprevalence of *Toxoplasma gondii* antibodies	Seroprevalence of *Toxoplasma gondii* antibodies by using ELISA		
	Cancer patient	Control group	Chi-square and *P* value
IgG (+) IgM (−)	11 (15.7%)	3 (15%)	NS
IgG (−) IgM (+)	4 (5.7%)	1 (5%)	Chi-square = 0.549
IgG (+) IgM (+)	7 (10%)	1 (5%)	*P* value = 0.908
IgG (−) IgM (−)	48 (68.6%)	15 (75%)	
Total	70 (100%)	20 (100%)	

IgG: immunoglobulin g, IgM: immunoglobulin m

Table 3: Results showing the number, gender, and age of participants and real-time PCR for *T. gondii* gene B1 detection

Groups	Number	Age	Gender	Samples with negative results of real-time PCR B1 gene (%)	Samples with positive+ results of real-time PCR B1 gene (%)
Cancer patients	16 (88.9%)	**10–70**	**6 males 10 females**	14 (77.8%)	2 (11.1%)
Controls	3 (11.1%)	**20–40**	**1 male 2 females**	3 (11.1%)	0
Total	**18 (100%)**			**16 (88.9%)**	**2 (11.1%)**

Around the world, the number of infections linked to cancer is rapidly increasing. Apicomplexan species like *T. gondii* are linked to neoplastic alterations in their host tissues; however, this association is controversial.[11] For patients with impaired immune systems, *T. gondii* has been identified as a significant opportunistic parasite infection.[12]

Despite the fact that the majority of *T. gondii* infections are clinically asymptomatic,[13] this parasite can result in serious illnesses, including fatal ones, especially in immunocompromised individuals, and also can lead to acute infection in immunocompromised people, as well as toxoplasmosis in newborns.

It has been known that both patients and controls infected with *T. gondii* showed a significant *P* value ($P = 0.052$); however, the rate of infection is different according to location, age, and other factors. In immunocompromised individuals, such as those with AIDS, cancer, organ transplants, or those using immunosuppressive medications, this disease is typically triggered by the reactivation of a latent infection; however, it can also result from an acute infection. Serological tests are typically utilized for the diagnosis of toxoplasmosis.[14]

IgG antibodies to toxoplasma are often detectable 1–2 weeks following the development of the infection and typically last for life. A 4-fold increase in the specific IgG titer and seroconversion with high quantities of toxoplasma-specific IgM are signs of recent infection in immunocompetent individuals.[15]

The real-time PCR assays detected *T. gondii* in cancer patients, as shown in Table 3, specific for gene B1 detection of toxoplasma to confirm ELISA assay, are shown in Table 3. In cases of acute infection or low-titer antibodies from illnesses that were just acquired, PCR is a direct method of identifying the parasite. It is a robust and very accurate diagnostic tool that can detect a single tachyzoite. It has been proven that assays for other targeted genes are less sensitive compared to a PCR-based approach for recognizing *T. gondii* DNA containing B1 repetitive sequences.[16] A PCR assay requires the presence of DNA to function; therefore, it has been pointed out that a large percentage of positive results for the B1 gene PCR may be due to the presence of the parasite's DNA rather than an actual infection.[17]

Conclusion

This is a crucial statistic that can save lives where parasite infections are widespread and cancer rates are rising, such as in developing nations. We can reduce the rates of infection-related fatalities by screening for infections and increasing public health awareness of the prevention of these infections.

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Conflicts of interest

There are no conflicts of interest.

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