

Toxoplasma gondii Could be a Problem in Diagnosis Scope? Current and Previous Diagnosis: A Narrative Review

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Received:2018/11/22 Accepted: 2019/03/10 Published: 2019/6/15

Abstract

The protozoan parasite *Toxoplasma gondii*, ubiquitous and intracellular organism, proposed as an important factor in patients with malignancy and immunodeficiency. Parasite infection has two phases in the clinical course. In the acute phase, in healthy people, only some common and mild clinical signs can be seen. However, in the chronic phase, parasite encysts in the brain of human and animal hosts and can reactivate after medical and immunological issues, which could be fatal. This narrative review discusses *T. gondii* diagnostic methods that performed routinely in medical and research laboratories and institutes. In this study, the main methods of most cited articles that evaluated *T. gondii* diagnostic methods were included. Traditional diagnosis methods of *T. gondii* include serology, animal inoculation, and live- parasite based test. Newly developed tests are polymerase chain reaction-based molecular and imaging methods. Some other methods that performed, e.g., antigen detection in body fluids, *toxoplasma* skin test, and antigen-specific lymphocyte transformation can be implemented according to the phase of the disease and available facilities.

Keywords: *Toxoplasma gondii*, Diagnosis, Epidemiology

Introduction

Toxoplasma gondii is a parasite that commonly infects cats as the definitive host, but it is also carried by a broad spectrum of warm-blooded animals, including humans. In some regions, up to one-third of the population is chronically infected with *Toxoplasma*, although most of the patients are asymptomatic (1, 2). However, women who become infected during pregnancy can pass the parasite to their unborn fetus according to the time of infection in pregnancy course. This can result in serious health problems for the fetus and newborns such as blindness and brain damage. In immunocompromised people, such as individuals infected with HIV or transplanted organs are also at risk of serious complications due to reactivation of dormant parasitic cysts in the brain. *Toxoplasma* parasites often actively invade host cells to multiply and survive. During an infection course, this multiplication is synchronized, means that all parasites in the host cell

replicate at the same time (2, 3). In North America and Europe, strains belonging to the types I, II, and III haplogroups are dominant, while in South America a large vast of other regions atypical strains are the incident agents. These strains differ grossly in virulence in laboratory animals (preferably mice), and there is evidence that different strains can also make variable pathogenesis in humans. In mice, the cytokine interferon gamma (IFN γ) induces multiple anti *toxoplasma* mechanisms and therefore plays some critical roles in immunity to *Toxoplasma*. Compared to mice, humans are more resistant to *Toxoplasma* (3, 4). In Iran, studies indicate that ocular toxoplasmosis related to type I. Genotyping of the *Toxoplasma* isolated from infants with congenital toxoplasmosis in Poland showed that type II, as the dominant type of *T. gondii* infection in congenital toxoplasmosis (5). In recent years, major efforts have been made toward improving the ability of detection newly acquired toxoplasmosis in the pregnant women and congenitally infected fetus and newborn. We now have

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some newly developed methods that are proving their worth for this purpose. For example, the serum IgG avidity test, polymerase chain reaction (PCR) on body fluids and tissues, and Western blots of serum from mother-baby pairs. Although our focus is on pregnant women and immunodeficiency patients, these methods are finding extensive use in the other groups of patients (6).

Materials and Methods

Search Strategies and databases

The current research was performed in databases without time limitation using such terms as follows: “*T. gondii*,” “meat,” “tissue cyst,” “PCR,” “loop-mediated isothermal amplification,” “screening” and “immunological assay” alone or in combination, in the English language. The electronic databases for searching included PubMed, Scopus, Google Scholar, Web of Science, and Science Direct. The searches were limited to the published papers to the English language.

1. Traditional diagnosis

1.1. Microscopic detection

The detection of *T. gondii* oocysts in fecal, water, environmental, and tissue samples has traditionally relied on microscope examination. However, identification based on light microscopy alone is less sensitive and unreliable. The oocysts form of the parasite in fecal samples, water resources, and environment can be enriched from large volumes of samples by filtration or centrifugation for examination, and the tissue cysts can be stained, which helps to distinguish the parasites from host cells. Giemsa and Haematoxylin and Eosin (HE) staining are simple, cost-effective, and commonly used for this purpose (7, 8). Another stain that can mark bradyzoites in tissue cysts is periodic acid Schiff that mixes with amylopectin in granules (9).

1.2. Live parasite-based diagnosis

The dye test or Sabin–Feldman dye test is a complement-based method and is the international gold standard for diagnosis of toxoplasmosis, but it is performed only in reference and research laboratories because live virulent *T. gondii* is used for the test. The dye test evaluates principally IgG antibodies and is both sensitive and specific. Since IgG antibodies persist in the dormant stage of the infection, detection of these antibodies in a single sample does not provide enough information about the timing of the initial infection or disease manifestation (10, 11). If *Toxoplasma* antibodies are present in the serum because these antibodies are activated by complements and lyses the parasite membrane, *Toxoplasma* trophozoites are not stained (positive result); if there are no antibodies, trophozoites with intact membrane absorbs the dye and appear blue under the microscope (negative result).

1.3. Animal lab parasite isolation

Mice peritoneal cavity is a suitable and easy region for parasite inoculation, and isolation after four days to one week. INF-gamma knockout mice are preferred, due to high sensitivity to *T. gondii* infection, or we can suppress the immune system of healthy mice by dexamethasone (12).

1.4. Serology methods

Toxoplasma infection usually is benign or asymptomatic, and the clinical sign and symptoms are not so clear. A vast spectrum of tests is available including modified agglutination test (MAT), enzyme-linked immunosorbent assays (ELISA), immunosorbent agglutination assay (ISAGA), indirect fluorescent antibody test (IFAT), and indirect haemagglutination assays (IHA), that have been implemented to detect different antibody types or antigens. IgM, IgG, and IgA antibodies are keys to detect infection and determine the stages of the disease. The shorter period of IgE antibody may indicate acute phase infection. The presence of IgG antibodies suggests the chronic phase of infection but does not provide information about the correct time of infection (9).

2. Newly developed methods

2.1. Nanomaterials

Nanomaterials like gold, nickel, magnetic, and quantum particles are available to detect *Toxoplasma*. Using 15 nm gold particles conjugated with Staphylococcal Protein A (SPA) used in cats and dogs as a serologic diagnosis of IgG (13). Time for gaining results in this method is shorter than ELISA. For isolating IgM, electrochemical immunosensor using magnetic gold nanoparticles method developed (14).

2.2. Cell and tissue culture

Vero, HeLa, Hep2, MRC5, human foreskin fibroblast (HFF), and many other cells can be the host for tachyzoites of *T. gondii* (15). In vitro culture of *T. gondii* can provide tachyzoites, which are active, viable, and with desirable purity. The harvested tachyzoites from mice, with desired cell counts, inoculates to various cell lines. The parasite multiplies in each cell, supernatant fluid of culture will be removed, and parasites are harvested. Automated cell counters and Neubauer chamber can be used to detect the number of tachyzoites (16).

*2.3. The high yield cells for *Toxoplasma* cultivation*

Large amounts of *T. gondii* can be harvested from human larynx carcinoma cell culture (Hep-2, heteroploid). These cells produce high yields of parasites (17). The Hep2 and Madian Darby Bovine Kidney (MDBK) cell lines were the most suitable for in vitro multiplication, followed by that of African green monkey kidney cells (VERO), pooled kidney from 1-day-old hamster (BHK), rabbit kidney cells (RK13) and human rhabdomyosarcoma (RDA), while chicken embryo cells (CER) were the least suitable (18).

*2.4. Primary culture of intestinal epithelial cells as a potential model for *T. gondii* enteric cycle studies*

Primary cultures of feline intestinal epithelial cells can be obtained from the fetus of a clinically healthy pregnant domestic cat (no gastrointestinal disease, negative for feline immunodeficiency virus and feline leukemia virus). The experimental strategies implemented in the present work reproduced in vitro the natural microenvironment established during enteric development of *T. gondii* in the definitive host, the domestic cat. Introducing the feline epithelial intestinal cell (FEIC) as a cellular model showed that it could potentially contribute to new ways to investigate the cell biology of the parasite. Also, FEIC is an alternative methodology to better understanding the enteric cycle of *T. gondii* under controlled conditions, opening up the field for investigation of the molecular aspects of this interaction and contributing, for example, to the development of new strategies aimed at intervention in one of the main routes by which toxoplasmosis spreads (19).

3. Molecular-based detection methods

3.1 Conventional PCR

Molecular detection methods like PCR are becoming new fast-growing fields in the diagnosis of parasites because of limitations in conventional diagnostic methods like simple microscopy due to the resemblance in their morphology. Several PCR assays have been introduced to detect *T. gondii* by targeting B1 repetitive gene, rDNA, P30 gene, and internal transcribe spacer (ITS-1) (20). PCR can be useful in the acute phase of the disease. PCR is much more precious in the diagnosis of cases that showed *Toxoplasma* parasitemia while Tox-IgG based ELISA can determine the *toxoplasma* specific antibodies, but it is unable to verify the acute toxoplasmosis (21).

3.2. Real-time PCR

Real-time PCR-based assay quantifies PCR amplified product with fluorescent technology. This method quantifies the products of the PCR reaction. The sensitivity of real-time PCR was 95.5% with a probe targeting the repetitive B1 gene of *T. gondii* to detect and quantify from human peripheral blood. The real-time PCR assay using FRET protocol by targeting a 529 bp repeat

region is considered as more sensitive and specific for *T. gondii* from immunocompromised patients and pregnant women as compared to real-time PCR based on TaqMan protocol, which targeted 18S RNA gene, and to nested PCR that targeted B1 gene of *T. gondii* (22).

3.3. LAMP

The LAMP, an attractive DNA amplification method developed as a valuable tool for the rapid detection of *T. gondii* via a sequence of 200- to 300-fold repetitive 529 bp fragment of *T. gondii*. The procedure of the LAMP assay is very simple, as the reaction would be carried out in a single tube under isothermal conditions at 64°C and the result would be read out with 1 h (as early as 35 min with loop primers). Thus, this method has the advantages of rapid amplification, simple operation, and easy detection and would be useful for rapid and reliable clinical diagnosis of the acute phase of toxoplasmosis, especially in developing countries (23). Figure-1 shows the color changes in positive cases.

Discussion

Diagnosis of *T. gondii* infection is based on serological tests, molecular methods, e.g., PCR, the examination of histopathologic specimens, and isolation of *Toxoplasma* by mouse peritoneal inoculation. However, most epidemiological studies used serological tests for evaluating *Toxoplasma* prevalence because they are more convenient to perform, less invasive, and usually present similar performance compared to other reference tests. Sabin-Feldman Dye test is generally regarded as the gold standard for diagnosis of *Toxoplasma* infection and is one of the most widely used tests for screening *Toxoplasma* infection in ovine and other animals (24). People most at risk of developing clinical symptoms include immunocompromised patients, pregnant women who acquire or have a reactivation the infection during the pregnancy, fetuses that congenitally or vertically infected, and individuals who have previously been infected in the uterus during pregnancy. Factors that increase the risk of acquiring a *T. gondii* infection include keeping a cat as pet, eating undercooked or raw meat, and poor personal hygiene. Therefore, various



Figure 1. Isolation of DNA samples of *T. gondii* and other parasites by the LAMP assay. Tubes of the LAMP reactions were visually inspected. Positive reactions turned green after the addition of SYBR Green I.

diagnostic methods can be useful in the detection of different forms of the parasite. Serological diagnosis can be difficult in prenatal cases or patients with immunodeficiency. Implementation of molecular diagnostic methods is particularly appropriate for such patients, as these techniques do not depend on the immunological status of the host. Although the immune responses play crucial roles in the exacerbating of toxoplasmosis, several studies have declared that the consequences and clinical presentations are also related to the virulence of specific genotypes of *T. gondii* (25). Currently, researchers used the rhoptry recombinant antigen to detect IgG in the acute phase. They showed that this antibody emerges in the acute phase at the early stage of the disease but not diagnostic in the chronic phase (26). The major advantage of using recombinant antigens for diagnosis of the infection is the ability to combine antigens in a single detection kit. Also, the use of recombinant antigens leads to better standardization of testing and reduce the cost of production. These considerations are very important when only one serum sample is available from a suspected patient (27). In the field of cell culture, we can evaluate drug efficacy on cell lines or any cell that can be a host of the parasite. Cellular cultures are also used for research purposes, like the study of host-parasite interaction, identification of factors involved on innate resistance, molecular and genetic characterization of *Toxoplasma* strains and for the evaluation of candidate molecules on vaccine and treatment development (28). The presence of *T. gondii* in a biological sample can be diagnosed by molecular techniques aimed at detecting its genetic material. A specific fragment of the genome can be amplified by PCR so that it can be visualized on agarose or a polyacrylamide gel following staining, on an automated sequencer by laser detection, or directly as an amplification product through real-time PCR techniques. The sensitivity and specificity of PCR-based methods depend on an appropriate technique for isolation of genetic material from samples, the characteristics of the DNA sequence chosen for amplification, and the parameters of the amplification reaction itself (29). Unfortunately, it is difficult to have confidential valor in the PCR assays. In patients with HIV infection, the detection of *T. gondii* by PCR in cerebrospinal liquid has shown different sensibilities, reaching to 87% of sensibility when the sample is taken soon enough preferably during the first three days and a maximum of seven days after starting the specific treatment (30).

Conclusion

As yet, there are no clear recommendations for routine tests that could be applied in case of *toxoplasma* diagnosis. According to the stage of the disease and available facilities, various tests may be implemented. Although serological tests are very common in hospital

and research laboratories, PCR method has high sensitivity and specificity, especially in immunocompromised hosts and in congenitally infected fetuses.

Acknowledgment

We would like to appreciate our colleagues in Parasitology Department of Hormozgan Medical faculty for their contributions. This study approved in Ethics committee of research in code of IR.HUMS.REC.1398.122.

Conflict of interest

The authors declare that they have no conflict of interest.

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