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Major Serological Screening Methodologies to Anti- *T. gondii* Antibodies Detection in Pregnancy: Mini Literature View

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Abstract

Congenital toxoplasmosis is a neglected protozoonotic disease of intercontinental distribution with incidence estimated in 1.5 cases per 1000 live births. This infection can be acquired intrauterine when the first infection of pregnant woman occurs and the *Toxoplasma gondii* reaches the fetus by transplacental via. The damage to the fetus can compromise the pregnancy and life quality of the infected individual. Early diagnosis of infection allows emergency therapeutic intervention with reduction of the transmission rate and also the severity of the sequelae. Different methodologies are available for the diagnosis of acute gestational toxoplasmosis with detection of IgG and IgM anti-*T. gondii* antibodies. The methodologies differ mainly by specificity and sensitivity values reached, as well as execution facility and time required. These parameters can decisively influence the choice of the most appropriate technique by the municipal health polices, in public health strategies. The main of the present review is to synthesize the major characteristics of serological tests for congenital toxoplasmosis.

Keywords: Congenital toxoplasmosis; Gestational toxoplasmosis; Public health; Serology

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Introduction

Toxoplasmosis is a protozoonosis worldwide distributed; its etiologic agent is *Toxoplasma gondii* (*T. gondii*), a protozoan belonging to the phylum Apicomplexa, class Sporozoasida, order Eucoccidiorida, family Sarcocystidae, genus Toxoplasma and single species, *T. gondii* [1]. The parasite exhibits a heteroxene cycle, with sexual reproduction in the intestines of felids, and asexual reproduction in all warm-blooded animals, such as birds and mammals, which act as intermediate hosts [2].

The asexual cycle begins with the ingestion of oocysts that have been eliminated by the feces of the infected cats or tissue cysts containing bradyzoites present in the raw or uncooked meat of the intermediate hosts [3]. After ingestion, the external wall of the oocysts or tissue cysts is ruptured by enzymatic degradation and the infective, sporozoite and bradyzoite evolutionary forms (slow-growing forms), respectively, are released into the intestinal lumen where they rapidly invade the host cells and differentiate in tachyzoites (forms of rapid multiplication) by asexual division [4]. The tachyzoites can invade any nucleated cell and form a parasitoid vacuole, where they undergo rapid and successive divisions by endodiogenia, originating new tachyzoites, which break the parasitized cell and invade healthy cells, which characterizes this phase as proliferative [5].

The dissemination of this parasite in the organism occurs through free or intracellular tachyzoites through the lymph, or blood, and can give rise to a polysymptomatic picture, with varying severity according to the parasite load, the parasite strain and the susceptibility of the host [6].

The frequency of infection may vary according to age, eating habits, cultural patterns and the urban or rural origin of the individual. The prevalence can vary between parts worldwide, with higher taxes in Latin America, parts of Eastern/Central Europe, the Middle East, South-East Asia and Africa [7]. The infection can occur by the ingestion of food or water contaminated with oocysts, or ingestion of undercooked or raw meat with cysts bradyzoites, or the tachyzoites by transplacental

via, nevertheless, the human toxoplasmosis has been associated with low socioeconomic conditions [8].

This increased risk may be due to changes in the placenta. In early pregnancy, the placental barrier reaches a thickness of 50 to 100 μm and progressively decreases to 2.5-5 μm at the end of pregnancy, allowing parasites to more easily invade trophoblasts.

According to the gestational age on the first infection with *T. gondii*, the damage to the fetus can vary due to the morphogenesis phase when affected by the parasite, therefore, in 80.0% to 90.0% of infections in immunocompetent individuals there is no clinical manifestation; however, when there is the infection in the first trimester of gestation, fetal death is common, and when in the second and third trimesters of gestation it may lead to prematurity and Sabin's tetrad, which associates microcephaly, retinocoriditis, cerebral calcifications and mental deficiency [9]. In 50.0% of congenital infections, fetal alterations such as hydrocephalus and periventricular necrosis with macro or microcephaly are reported. In 90.0% of patients there is marked retinal destruction and retinocoriditis, in 69.0% cerebral calcifications are developed and in 60.0% of the cases are generated mental retardation or neurological disorders with signs of encephalitis and with seizures [10].

The chance of congenital infection is estimated between 4.5% and 15.0% when there is toxoplasmosis in the first trimester, followed by 17.3% to 30.0% in the second third, and 60.0% to 75.0% in the last period [10,11]. This increase in the rate of infection according to gestational age is due to the morphological changes of the placenta, because in the first trimester of pregnancy, the placenta has reduced dimensions and the chance of the parasite reaching the fetus is small, in contrast, as in this phase happens the organogenesis, when there is differentiation of the germinative leaflets, ectoderm, mesoderm and endoderm giving rise to the internal organs of the organism, aggravating the infection, that can lead to fetal death [12].

In the second trimester, increased placenta reflects a greater chance of infection, however, in this period, the infection becomes less severe, since almost all organogenesis has already occurred, meantime, it can result in important damages such as Sabin's Tetrad, in which the fetus presents retinocoriditis, cerebral calcifications, mental retardation or neurological disorders and hydrocephalus, with macro or microcephaly [9]. In the third trimester, the placenta presents the largest dimensions of the period, consequently, the chance of infection is higher and, in contrast, the chance of developing serious damage at birth is remote, with manifestations of the disease such as fever, body spots, blindness, in a few days, weeks or months after birth [12].

The reduction in incidence, as well as morbidity and mortality rates associated with congenital toxoplasmosis can be achieved in the presence of collaborative efforts among pregnant women, researchers, doctors and health policy managers, on the adoption of primary and secondary health preventive measures in the gestation [13].

The diagnosis of acute *T. gondii* infection in the gestational period is crucial to define the risk of fetal involvement [14]. For this, prenatal care should be carried out continuously from the gestational diagnosis, in order to avoid congenital toxoplasmosis and also the other risks associated with the life of the pregnant woman and the fetus [15]. In Brazil, health professionals who work in the care of pregnant women who perform prenatal care through the Unified Health System (SUS), comply with the protocols established by the Ministry of Health, which guide the serological monitoring for primary infection by *T. gondii* [16]. Confirmation of toxoplasmosis is based on indirect or immunological methods, since the direct demonstration of the protozoan by culture, inoculation, immunohistochemistry and pathology is in frequent [17].

The molecular techniques with direct detection of *T. gondii* tachyzoite is possible during the acute phase of infection and can confirm fetal infection from different body fluids such as blood, amniotic fluid, cerebrospinal fluid, aqueous humor, bronchial lavage fluid alveolar and even urine by PCR technique with a sensitivity of up to 92.0%, which makes this the method of choice in cases of low parasitemic, on the other hand, in the chronic phase of infection, as in cases of cysts in brain or pulmonary tissue, the technique will not be useful [18].

Indirect or serological techniques, through the detection of antibodies of the IgM, IgG, IgE and IgA classes, do not require invasive procedures that pose a risk to the fetus such as amniocentesis and can be performed at any time during pregnancy. Several methods are available for the serological diagnosis of gestational toxoplasmosis such as the Sabin-Feldman technique, Immunofluorescence antibody test (IFAT), Hemagglutination (HA), complement fixation (FC), enzyme-linked immunosorbent assay (ELISA), immunoblotting and Imunosorbent Agglutination Assay (ISAGA), with differences between the values of sensitivity and specificity reached [19].

It is important that the serological tests for specific anti-*T. gondii* antibodies are performed at the first prenatal visit with serial analysis in the second and third trimester of gestation and the pregnant woman should receive guidance on the preventive measures of infection [10].

In the serological diagnosis, specific IgM class antibodies are usually the first to be detected, whereas IgG antibodies appear later, with high titers during the acute phase and gradual reduction with persistence of low titers in the majority of cases, throughout the individual's life, both in the latency phase and in the chronic phase. IgM antibodies may be detectable for long periods, months to years after the acute phase of infection only by highly sensitive diagnostic methods. However, when this antibody is not detected by traditional techniques, or inability to observe IgG seroconversion during pregnancy, the expression of increased IgG titers in serial samples obtained at least three weeks apart, and in tests may suggest the diagnosis of the acute phase of *T. gondii* infection. Among the techniques for identification of recent *T. gondii* infection in pregnant women is the IgG and IgM capture ELISA [20].

In immunocompetent individuals, serological tests with IgG and IgM screening are sufficient for diagnosis because they are sensitive, specific and easy to perform [21].

Serological screening has several advantages, such as the identification of seronegative pregnant women, which provides the opportunity for health professionals to act in the preventive education of gestational and congenital toxoplasmosis, since eating habits and personal hygiene practices are identified as factors associated with the risk of infection. On the other hand, the identification of asymptomatic patients allows the establishment of adequate therapeutic measures, while the early detection of maternal seroconversion during pregnancy makes it possible to monitor and treat fetal infection and newborns [22].

The mechanisms by which parasites overpass the maternal-fetus interface remains poorly understood and may reinforce the

importance of the screening serological tests to a successful gestation and to minimize the outcomes in the pregnancy due to congenital or gestational toxoplasmosis [23].

The main characteristics of the techniques used to diagnose *T. gondii* infection in prenatal care were the object of our study and are summarized in this mini review of qualitative literature, made up of scientific articles and books, available online, on the theme "serological diagnosis for gestational and congenital toxoplasmosis".

Major characteristics of the methods for anti-*T. gondii* antibodies detection

Sabin-feldman dye test: The Sabin-Feldman reaction or dye test was described by Sabin & Feldman, in 1948 and is a differential serological test, where there is specific neutralization of the viable parasite in the presence of antibodies and complement. This method is considered excellent for the diagnosis of individual toxoplasmosis in the acute or chronic phase and also for epidemiological surveys, on account of its high sensitivity, indicating IgG antibodies in serum dilutions up to 1:16000 and specificity with little cross-reaction with other diseases, however, due to the need to manipulate the parasite in its infecting form, is not used as first option for routine diagnosis. Currently, for biological safety, the technique has been replaced by Indirect Immunofluorescence which also has lower cost and also detects IgG and IgM antibodies [24]. Besides the conventional Sabin-Feldman dye test are the gold stand for human toxoplasmosis, the conventional test using *in vivo* tachyzoites is the highest percent of toxoplasmosis detection in human serum when compared the results when using cell culture-derived *T. gondii* tachyzoites and when test indirect immunofluorescent antibody tests (IFAT) using *in vivo* and *in vitro* tachyzoites.

The Microparticle Enzyme Immunoassay (MEIA) technique

The MEIA makes quantitative determination of IgG and IgM anti-*T. gondii* antibodies in human serum or plasma. The immunoassay works on a random and continuous way in an Abbott company machine (AXSYM), and for the IgM antibodies detection a rheumatoid factor neutralization solution is required to remove no specific antibodies avoiding false positive results. At the end of the reaction, the immune complex bound to the alkaline phosphatase-labeled conjugate reacts with the substrate to generate fluorescence of intensity proportional to the antibody concentration of the analyzed sample [25].

The Enzyme Linked Immunosorbent Assay (ELFA)

The ELFA is an automated test in the VIDAS system of the company Bio-Mérieux, used for IgG and IgM antibodies anti-*T. gondii* detection, through the immunoenzymatic method with a final fluorescence detection as the main reaction, named immunocapture, where the IgM antibodies present in the immunocomplexare labeled with alkaline phosphatase. This method, when compared to the ISAGA (Immunosorbent Agglutination Assay), demonstrated agreement of 98.9%, sensitivity of 93.5% and specificity of 99.3% [26].

The Indirect Fluorescent Antibody Test (IFAT)

IFAT is indicated for serological surveys and diagnosis of acquired infection, because it can reach sensitivity up to 95.0%, however, false positive reactions with antinuclear factor (FAN) and false negative in the presence of low IgG titers can occurs [27].

Enzimed Link Immunosorbent Assay (ELISA)

The ELISA is an immunoenzymatic assay can reach up to 80.0% of positivity and identifies early infection, with special importance for the diagnosis of congenital toxoplasmosis. The technique eliminates the interference of IgG and rheumatoid factor that are present in IFAT and can be standardized for detection of different classes of antibodies. When ELISA is used for IgG detection, a high positivity index has been observed in the Brazilian population, however, it is not possible to predict whether the infection is acute or chronic [28].

In regions were the cost of the kits is expansive for screening proposes, some in-house ELISA for the detection of toxoplasma antibody demonstrated sensitivity and specificity as the commercial kit used in the study with a better cost-effective diagnostic [29]. To detect recent infection, the research is directed to the identification of IgA, which remain with high titers for at least 24 months and, because they do not cross the placenta and are not eliminated through breast milk, increase the importance to the use of this technique for the diagnosis of toxoplasmosis in the newborn [30].

The ELISA is available as commercial kits and requires an Analyzer for the detection of specific IgG against *T. gondii*. Briefly, the serum-specific IgG against the parasite is linked to *T. gondii* coated antigen on the surface of reagent wells, forming an antigen-antibody complex. The goat anti-human IgG labeled with horse radish peroxidase can be used as the secondary antibody to the antigen-antibody complex and after incubation and wash, as a chromogenic substrate is added to each well. The process should be stopped using a stop solution of 1 N of H_2SO_4. The Optical Density (OD) is recorded at 450 nm in the analyzer equipment. Calibrator and positive/negative control sera are used in each test set and the immune status ratio value of each sample is calculated by dividing OD of the sample by the cutoff value (cutoff is the mean OD of the calibrators x correction factor). Results were interpreted based on the manufacturer's recommendations as non-reactive, equivocal, or reactive [31].

The ELISA (for the qualitative determination of specific IgM antibodies anti *T. gondii* in dry blood samples collected on filter paper for in vitro diagnostic use only), MEIA and ELFA have been used to improve the diagnosis of toxoplasmosis, which has demonstrated to be useful for the diagnosis of toxoplasmosis, as they show greater sensitivity and specificity [32].

The IgG avidity test

IgG Avidity concerns on the observation that, in most patients, during acute *T. gondii* infection, IgG antibodies bind poorly to the antigen that is low avidity, whereas in the chronification of the infection, the bond strength increases and high avidity can develop. These antibodies with high avidity reflect the fact that the primary infection occurred in the distant past, estimated about more than three months, although this method cannot determine if the infection was recent, since eventually low titers can persist for long periods, such as after antiparasitic treatment, which can maintain low avidity for more than four months. Thus, the test is especially indicated for the first trimester of gestation in IgG and IgM reagent women for screening serology, when it was shown to be the best marker of acute infection in patients with positive IgM [27].

The Immunobloting Test

Immunoblotting is performed by segregation of the protein antigens components through the polyacrylamide gel electrophoresis. The proteins are transferred to a nitrocellulose paper and processed against the serum to be tested, which, in the presence of reaction, can be visualized. The test is used to determine antigens involved in the

different stages of infection, however, it is not a routine test [19].

Conclusion

The serological screening for gestational toxoplasmosis is essential for the identification of susceptible pregnant women and fast detection of IgM seroconversion, with monitoring since the first trimester of pregnancy for the efficient prevention of congenital toxoplasmosis. Among the techniques for detection of anti-*T. gondii* antibodies in pregnant women, the sensitivity and specificity of antibodies, verified by MEIA, ELFA and ELISA techniques were higher when compared to IFAT detection. The recent infection in the first trimester of gestation should be confirmed by the avidity test.

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