REVIEWS

CONGENITAL TOXOPLASMA GONDII INFECTION

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ABSTRACT

Pregnant women who acquire infection from Toxoplasma gondii usually remain asymptomatic, although they can still transmit the infection to their fetuses with severe consequences. Transmission of T. gondii to the fetus can result in serious health problems, including mental retardation, seizures, blindness, and death. Some health problems may not become apparent until the second or third decade of life. Serologic tests are used to diagnose acute T. gondii infection in pregnant women. Because false-positive tests occur frequently, serologic diagnosis must be confirmed at a Toxoplasma reference laboratory before treatment with potentially toxic drugs is considered. In many instances, congenital toxoplasmosis can be prevented by educating pregnant women and other women of childbearing age about not ingesting raw or undercooked meat, using measures to avoid cross-contamination of other foods with raw or undercooked meat, and protecting themselves against exposure to cat litter or contaminated soil.

Keywords: Toxoplasma gondii, Congenital infection, Diagnosis, Treatment, Follow-up

KONJENİTAL TOXOPLASMA GONDİİ ENFEKSİYONU

ÖZET

Hamile kadınlar Toxoplasma gondii enfeksiyonunu genellikle asemptomatik olarak geçirmelerine rağmen bebeklerini enfekte ederek onlarda bu enfeksiyona bağlı ciddi sekellere neden olurlar. T. gondii enfeksiyonu fetusda mental retardasyon, havale, körlük ve ölüm gibi ciddi sağlık problemleri ile sonuçlanır. Bu sağlık problemlerinden bazıları 20`li ve 30`lu yaşlara kadar ortaya çıkmayabilir. Gebede akut T. gondii enfeksiyonu tanısında serolojik testler kullanılır. Bu testlerde sıklıkla yalancı pozitif sonuçlara rastlanılır. Bu nedenle potansiyel toksik etkileri olan ilaçlarla tedaviye baslamadan önce mutlaka pozitif serolojik test sonuçları referans laboratuvarında doğrulanmalıdır. Çoğu durumda konjenital toksoplasmosis hamile kadınların ve diğer doğurganlık yaşında olan kadınların çiğ veya pişmemiş et ürünleri tüketmemeleri, bu tür gıdalarla kontaminasyondan kaçınmaları ve kontamine toprak veya enfekte kedilerin dıskı kaplarına maruz kalmaları durumlarında kendilerini korumaları konularında eğitilmeleri ile önlenebilir.

Anahtar Kelimeler: Toxoplasma gondii, Konjenital enfeksiyon, Tanı, Tedavi, İzlem

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INTRODUCTION

Virology

T. gondii, a member of the phylum Apicomplexa, order Coccidia (all obligate intracellular protozoan parasites), has been defined in three forms existing outside the cat intestine: oocyst, *endozoite or tachyzoite and cystozoite or bradyzoite*[1].

Oocysts are produced as part of sexual reproduction in the small intestine of a cat that has recently ingested tissue cysts, usually by consuming uncooked meat. Almost two weeks after the initial infection, oocysts, which contain infective sporozoites, are produced in the cat. One to 5 days after they are produced and deposited, they become infective[2]. Tachyzoites, the rapidly dividing products of asexual reproduction in macrophages following invasion of the host intestinal wall by either sporozoites or bradyzoites, are disseminated hematogenously by macrophages in an intermediate host until an adequate immune response occurs after 7 to 10 days. With immune response development, the protozoan is contained within tissue cysts as bradyzoites, or slowly dividing *T. gondii*. Although these tissue cysts can remain dormant for the lifetime of the intermediate host in various tissues, including the lymph nodes, muscle, brain, retina, myocardium, lungs, and liver, bradyzoites can continue their rapid division and hematogenously disseminate in the form of tachyzoites again, particularly in immune compromise, such as with the use of immunosuppressive therapy or acquired immunodeficiency syndrome[3].

Pathogenesis

The course of infection in human beings and animal models of toxoplasmosis seems to be affected by inoculum size, virulence of the organism, sex, and immunological status. Genetic background has been reported to play an important role in increased susceptibility to *T. gondii* in humans; HLA-DQ3 has been found to be a genetic marker associated with susceptibility to developing toxoplasma-dependent encephalitis[4,5].

With its ability to induce a strong cellular immune response in the normal host, toxoplasmosis is an opportunistic infection. Thus, cell-mediated immunity is essential for host resistance to the parasite[6]. After oral ingestion of the parasite, intestinal epithelial cells are actively invaded or they phagocytose the parasite[7]. Migrating over long distances in the host's body, *T. gondii* has been shown to cross biological barriers, actively enter the blood stream, invade cells and cross substrates and non-permissive biological sites such as the blood-brain-barrier, the placenta and the intestinal wall. It also minimizes exposure to the host's immune response, by rapidly entering and exiting cells, the mechanisms of which are common and depend on Ca2+ regulation[8].

T. gondii, an obligatory parasite with invasive capabilities acting in virulence and pathogenicity, can only survive intracellularly where it gets nutrients and escapes from the host's immune response[9]. The most virulent *T. gondii* strain with superior migratory capacity has been shown to have a subpopulation with a special, long distance migration phenotype[10].

Infection with *gondii* results in a strong and persistent T-helper-1 (Th1) response characterised by production of proinflammatory cytokines including interleukin 12, interferon γ, and tumour necrosis factor α. The host is protected against rapid replication of tachyzoites and subsequent pathological changes by the combined action of these cytokines and other immunological mechanisms[7]. IgG, IgM, IgA, and IgE antibodies against many *T gondii* proteins can be detected within 2 weeks of infection. IgA antibodies produced on mucosal surfaces seem to protect the host against reinfection[11,12]. Although reinfection can occur, it does not seem to result in disease or in congenital transmission of the parasite[7].

Epidemiology

The most frequent causes of *T. gondii* infection are ingestion of raw or undercooked meat containing tissue cysts, consumption of

infected water or food, or accidental intake of contaminated soil. Toxoplasmosis has been shown to be an occupational hazard for laboratory workers[1]. In the nation-wide data presented in the study by Tender et al, the rates of positive sero-prevalence for *T. gondii* in women of child-bearing age (1990–2000) were 58% in Central European countries, 51–72% in several Latin-American countries, and 54–77% in West African countries[13]. The rate for southwest Asia, China, and Korea was as low as 4–39%, whereas it was even lower in cold climate areas such as Scandinavian countries (11–28%). The rate for the US was 15% in females of child-bearing age[14]. In Turkey, Aslan et al studied the distribution of anitoxoplasma antibodies in women from different age groups. Seropositivity was found as 18.5% in the 15-25 year age group, 30.7% in the 26-35 year age group and 35.4% in the 36-45 year age group in the study[15]. Also, Güngör et al found that toxoplasma antibody positivity was 41.6% in 245 pregnant women who attended their laboratory for routine pregnancy controls[16]. Seropositive prevalence may differ even in the same country, among populations or geographical regions and world-wide prevalence is higher in older populations[1].

In the studies designed to determine the risk factors for *T. gondii* infection during pregnancy, various results were reported for Italy, Norway, and Yugoslavia[17-19]. Two of the studies compared pregnant woman who had recently seroconverted or who had evidence of recently acquired infection with seronegative matched controls. Another study from Yugoslavia compared seronegative with seropositive persons who had had past infection. The results of these four studies indicated that the factors associated with increased risk were ingestion of raw or undercooked meat, use of kitchen knives that had not been sufficiently washed, and ingestion of unwashed raw vegetables or fruits. A recent case-control study from Europe examined the risk factors that predisposed pregnant woman to infection with *T.gondii*[20]. In this study, approximately 30% to 63% of the infections were due to exposure to inadequately cooked or cured meat, which was interpreted to be the main risk factor for pregnant women in Europe. The other risk factors listed were contact with soil, accounting for approximately 6% to 17% of infections, and traveling outside Europe or the United States and Canada were the other risk factors listed. Direct "contact with cats" was not a risk factor, however, contact with soil would presumably reflect risk from cat excrement. Strikingly, the way of acquisition remained undefined for a large proportion of the infections (14% to 49%). In another recent study on risk factors, in the United States between 1981 and 1998, undercooked meat or possible cat excrement exposure, or both, were defined by 50% of the mothers of infants with congenital toxoplasmosis, but the rest of the mothers could not identify the risk factors[21]. A study from Turkey has indicated that ingestion of raw or undercooked meat and unwashed raw vegetables or fruits was associated with an increased risk for *T. gondii* infection during pregnancy[22].

It should be emphasized that in recent epidemiologic studies, cat ownership has not been shown to be a consistent risk factor for *T gondii* infection, rather it is related to being exposed to feces from a cat that is shedding oocysts. Since indoor cats do not hunt and are not fed raw meat, they are unlikely to acquire T.gondii infection, and therefore they pose little risk[23]. Testing a cat's stool to determine human risk does not aid because cats shed oocysts for only a short period of time. Furthermore, cats often do not develop antibodies to T. gondii during the oocyst-shedding period. Thus, serologic testing does not provide useful information about the ability of a particular cat to transmit toxoplasmosis[24].

Transmission

Lifelong immunity is gained after *toxoplasma* along with specific IgG antibodies. However, in pregnancy, primary infection of the mother may lead to vertical transmission, followed by fetal infection which presents the risk of congenital abnormalities. The time of maternal infection, immunological competence of the mother during parasitemia, parasite load and strain's virulence constitute

the basic factors for fetal infection[1]. The risk of fetal infection varies depending on time as only 1% at less than 6 weeks, 4–6% at 6–16 weeks, 20–40% at 16–25 weeks and 60–80% at 36 weeks of gestation[25].

Literature reveals only a few cases of congenital toxoplasmosis transmitted by mothers who were infected prior to conception[26,27,] for example the case of a woman who had ocular toxoplasmosis 20 years prior to giving birth to a newborn with congenital toxoplasmosis[28]. The mother, who had a "toxoplasmic scar" in the retina, was tested positive for specific toxoplasma IgG antibodies. The newborn was positive for both IgG and IgM antibodies and had a macular scar on the retina, which is typical to toxoplasmosis, as well as a calcified brain granuloma. This could be attributed to re-infection with a different, more virulent strain or by reactivation of a chronic disease[26]. The infection can also be transmitted to the fetus by chronically infected women who are immunodeficient, the risk of which though difficult to estimate, is probably low. Latent *T. gondii* infection may be reactivated in immunodeficient individuals (such as HIV-infected women) and result in congenital transmission of the parasite[29].

Clinical Manifestations

In more than 10% of adult patients, infection usually does not lead to specific symptoms. Fatigue, and in some women, a mononucleosis-like syndrome with fever, malaise, pharyngitis, headache and lymphocytosis may be seen[25]. The most common manifestation is lymphadenopathy in non-pregnant and pregnant individuals, causing 3-7% of clinically significant cases[30].

Prenatal ultrasound usually shows no abnormality in fetuses with congenital toxoplasmosis. When ultrasonographic findings are present, they suggest congenital disease including intracranial calcifications, ventricular dilatation, hepatic enlargement, ascites, and increased placental thickness[31]. Neonatal clinical manifestations of congenital toxoplasmosis are various, including hydrocephalus, microcephaly, intracranial calcifications, chorioretinitis, strabismus, blindness, epilepsy, psychomotor or mental retardation, petechia associated with thrombocytopenia, and anaemia[32,33]. The classic triad of chorioretinitis, hydrocephalus, and cerebral calcifications is very rare. Because the signs described in newborns with congenital disease are not pathognomonic for toxoplasmosis, they can be mimicked by congenital infections such as cytomegalovirus, herpes simplex virus, rubella, and syphilis due to other pathogens[7]. Despite invading all fetal tissues, toxoplasma gondii exists in proportionately larger numbers in the central nervous system, resulting in retinochoroiditis and intracranial calcification or ventricular dilatation, the most common clinical manifestations in 14 to 17% of infected infants[34]. In cases with suspected *T. gondii* infection at the time of birth, diagnostic ophthalmic, auditory and neurological examinations, lumbar puncture and cranial imaging are diagnostic tools[1].

Chorioretinitis, the most prevalent consequence of congenital toxoplasmosis, is diagnosed based on characteristic retinal infiltrates. Vutova et al investigated eye manifestations of congenital toxoplasmosis in 38 infants and children and reported that the most frequent finding was chorioretinitis (92%) as well as microphthalmia with strabismus[35]. An uncommon finding was the lesions of the segment of the eye including iridocyclitis, cataracts and glaucoma. Other uncommon findings were diminished visual acuity and neurological sequelae such as hydrocephalus, calcification in the brain, paresis, and epilepsy. Potasman et al studied 95 children with variable neurological disorders: cerebral palsy, epilepsy and nerve deafness for the presence of *T. gondii*-specific antibodies in the serum in a case–control study in Israel and compared their results with the results of 109 healthy children[36]. It was reported that children with any of the neurological disorders were significantly more likely to have *T. gondii* specific IgG antibodies, especially those with nerve deafness (relative risk 2.5 and 7.1, respectively).

Diagnosis

The diagnostic tools for *T. gondii* infection or toxoplasmosis are serologic tests, amplification of specific nucleic acid sequences (i.e., polymerase chain reaction [PCR]), histologic demonstration of the parasite and/or its antigens (i.e., immunoperoxidase stain), or isolation of the organism. Demonstration of antigenemia and antigen in serum and body fluids, a toxoplasmin skin test, and antigen-specific lymphocyte transformation are other rarely used methods[2].

Serologic Tests

The initial and primary method of diagnosis involves the use of serologic tests for demonstration of specific antibody to *T. gondii*[37].

IgG antibodies: Ig G antibodies usually appear within 1-2 weeks of acquisition of the infection and peak within 1-2 months; however, they decline at various rates and usually persist for life. Sabin-Feldman dye test (SFDT), ELISA, indirect fluorescent assay mmunofluorescent antibody test (IFA), IgG avidity test, and agglutination and differential agglutination test are the most commonly used tests for the measurement of IgG antibody[7].

As the first test developed for the laboratory diagnosis of *T.gondii* infection, SFTD is still considered the "gold standard" because it detects the presence of anti-*T. gondii* specific antibodies (total Ig) and is performed only in reference centers. Serum samples taken at least 3 weeks apart are important in determining the change in antibody titer for the evaluation of infection during pregnancy. At least a four-fold difference is considered a "significant" change. Moreover, the absolute antibody titer is also important—values over 250 IU/ml are considered "high" suggestive of recent infection[1].

Avidity (functional affinity) tests for IgG antibodies have become standard in discriminating recently acquired infection and those obtained in the more distant past[38]. Infection acquired in the recent 3–4 months is ruled out in the presence of high avidity antibodies, while low avidity antibodies can endure beyond 3 months of infection[39-41]. In combination with a panel of other assays, the differential agglutination (AC/HS) test has also proven helpful in discriminating a probable acute or chronic infection in pregnant women[37,42,43].

IgM antibodies: Although in patients with recently acquired primary infection, T. gondii specific IgM antibodies are detected initially, in most cases, these titers become negative within a few months. In some patients, however, positive T.gondii-specific IgM titers can still be found during the chronic phase of infection[43]. IgM antibodies have been detected as long as 12 years after the acute infection [44]. Even if the persistence of these IgM antibodies does not appear to have any clinical relevance, these patients should be considered chronically infected. The interpretation of a positive IgM test results in a relatively high frequency of false-positive results [43,45]. In other words, a positive IgM test result in a single serum sample can be interpreted as a true-positive result in the setting of an infection acquired in the distant past, or a false-positive result[37]. Double-sandwich or capture IgM-ELISA, the IFA test, and IgM immunosorbent agglutination assay (ISAGA) are the most commonly used tests for the measurement of IgM antibody.
IgA antibodies: Tests for the detection of IgA antibodies have been proven to be more sensitive than those used for detection of IgM antibodies in the fetus and newborn[7]. The serologic diagnosis in a number of newborns with congenital toxoplasmosis and negative IgM antibodies has been established by the presence of IgA and IgG antibodies [37]. ELISA and ISAGA can be used in the detection of IgA antibodies.

IgE antibodies: IgE antibodies are detected by ELISA in sera of acutely infected adults, congenitally infected infants, and children with congenital toxoplasmic chorioretinitis. However, unlike with IgA tests, their detection does not seem to be particularly useful for diagnosis of T. gondii infection in the fetus or newborn. IgE seropositivity lasts

less than that with IgM or IgA antibodies; thus, this appears useful for identifying recently acquired infections[37].

PCR

PCR amplification for detection of T.gondii DNA in body fluids and tissues has been successful in diagnosing congenital, ocular, and cerebral and disseminated toxoplasmosis[2].

Although influenced by different protocols, the specificity and positive predictive value of PCR tests on amniotic fluid samples is close to 100%[46,47]. However, the sensitivity of these PCR tests is 70–80%, based on a large number of studies[48]. In one report, the stage of pregnancy in which maternal infection occurs was shown to affect the sensitivity of PCR from amniotic fluid, with best sensitivity detected between 17 and 21 weeks of pregnancy when maternal infection occurred[47]. Furthermore, the use of anti-toxoplasma drugs in treatment may also affect the sensitivity[49]. However, further studies are required on the reliability of a PCR test performed on amniotic fluid prior to the 18th week of pregnancy.[46,47]. Another important point is that testing amniotic fluid for *T. gondii* was found to be effective about 4 weeks following infection, which is already during the parasitemic stage in the infected mother. Therefore, PCR test should not be performed in the absence of serologic or other clinical/sonographic data suggesting infection[1].

In the very recent past, Real Time PCR has been used and advocated as a sensitive and specific technique, enabling rapid detection of amplification products as well as hybridization of amplicon-specific probes, similar to PCR followed by Southern blot analysis. The Real-time PCR will probably be more commonly used in the future[7].

Histologic Diagnosis

The presence of tachyzoites in tissue sections or smears of body fluid (e.g., CSF or amniotic or BAL fluids) establishes the diagnosis of the acute infection. However, it is often difficult to demonstrate tachyzoites in conventionally stained tissue sections[2].

Isolation of T. gondii

A solid proof of infection is definitely the isolation of the parasite from an infant. Nevertheless, such isolation usually takes too long to permit an early diagnosis. The parasite can be isolated by mouse inoculation or inoculation in tissue cell cultures of virtually any human tissue or body fluid[37].
T. gondii Infection in Pregnancy As soon as acute T. gondii infection is suspected in a pregnant woman, the diagnosis should be established, usually on the basis of antibody detection. In acute infection, IgG and IgM antibody levels generally rise within one to two weeks of infection[2]. Elevated levels of *T. gondii*–specific IgG antibodies suggest that infection has occurred but does not differentiate recent infection from an infection acquired in the distant past. The time of infection has been determined based on the detection of T. gondii–specific IgM antibodies: a negative IgM test result with a positive IgG result indicating infection at least six months previously. However, the persistence of IgM antibodies up to 18 months after infection and by false-positive reactions in commercial tests have complicated the interpretation of *T. gondii*–specific IgM-positive results[45]. Thus, IgM-positive test results should be confirmed by a Toxoplasma reference laboratory, which may also be able to narrow the time of infection with specific tests (e.g., IgG avidity test) or a serologic profile (e.g., Sabin-Feldman dye test, IgM enzyme-linked immunosorbent assay [ELISA], IgA ELISA, IgE ELISA, differential agglutination)[23].

Congenital Infection in the Fetus and Newborn

Prenatal diagnosis

Once the diagnosis of acute maternal infection has been established, it should be determined whether the fetus is infected or not. Chorionic villus sampling (CVS) can only show placental but not fetal infection; thus, it is not

useful. Cordocentesis, however, has been the most widely used diagnostic test for the determination of fetal IgM status and mouse inoculation studies. Although non-specific, hematological and liver function can also be evaluated. Still, fetal blood sampling may not yield a reliable conclusion, possibly because of an immature fetal immune system, as fetal IgM or IgA antibodies may not be produced before 22 weeks of gestation[25]. Many previous studies have reported the low sensitivity of the serologic diagnostic tests on fetal blood[50-52]. In these studies, specific IgM antibodies were detected in 47–58% and IgA antibodies in 37–77% of cases. Another problem of fetal blood sampling is possible false-positive results because of the transplacental passage of non-viable *T.gondii* organisms following maternal therapy, which then could lead to the development of a fetal immune response but not active infection[63]. Among all the parameters evaluated, the PCR assay of amniotic fluid was found to be the most sensitive.

Amniotic fluid assessment using PCR is rapid and accurate; thus, it is now the procedure of choice for diagnosing fetal toxoplasmosis. When the concentration of the parasite in the amniotic fluid is low, DNA amplification may be the only positive result[25].

However, using PCR on the amniotic fluid of HIV-infected women is not recommended because of the risk of transmitting the HIV virus to the fetus during the amniocentesis procedure[37].

Diagnosis in the newborn Laboratory diagnosis of *Toxoplasma* infection in infants involves a combination of serologic tests, parasite isolation, and nonspecific findings[7]. In case of suspected infection, serologic follow-up of the newborn is recommended for the first year of life.

While maternal IgG antibodies present in the newborn may reflect either past or recent infection in the mother, passively transferred maternal IgG has a half life of approximately 1 month but can still be detected in the newborn for several months, generally disappearing completely within one year[1]. In an untreated patient, autonomous IgG antibodies in a congenitally infected newborn appear about 3 months after birth. Antibody production may be delayed by anti-parasitic therapy for about 6 months and occasionally may be completely prevented[2].

The detection of IgM or IgA antibodies to *T. gondii* in an infant is highly sensitive for the diagnosis of congenital toxoplasmosis, identifying 75% of infected babies[7]. Because serum amples from the umbilical cord may be contaminated with maternal blood, they should not be used. Serum samples obtained from peripheral blood are preferred.. Demonstration of IgA antibodies has been more sensitive than detection of IgM antibodies for establishing infection in the newborn[54]. *T. gondii*–specific IgA may be present when there is no *T. gondii*–specific IgM, and the vice versa may also occur. When IgA antibodies are detected in the newborn, the test should be repeated at ~10 days after birth in order to ensure that what is being measured is not contaminating maternal IgA antibodies[37].

In babies with suspected congenital toxoplasmosis with positive IgG but negative IgM and IgA tests results, use of IgG/IgM western blots of mother-infant pairs can prove useful[7]. Some other successful diagnostic methods to diagnose the infection in infants include direct demonstration of the organism by isolation of the parasite (e.g., Mouse inoculation or inoculation in tissue cultures of CSF, urine, placental tissue, or peripheral blood) and amplification of T. gondii–specific DNA (e.g., PCR in CSF, peripheral blood, or urine)[2]. In addition, infants with suspected congenital toxoplasmosis should always be evaluated through ophthalmologic examination, non-contrast computed tomography or ultrasound of the brain (to determine whether hydrocephalus or calcifications are present), and examination of CSF[37].

Antigen-specific lymphocyte transformation and lymphocyte typing in response to exposure to T. gondii antigens has also been successfully used to diagnose the congenital infection in infants older than 2 months of

age[55,56]. However, it is clinically unavailable. Specific lymphocyte anergy to the organism may also occur in congenitally infected infants[57].

Treatment

Treatment of the Fetus through the pregnant Woman

The effectiveness of prenatal treatment has been investigated by a limited number of retrospective cohort studies that focused on gestational age at maternal infection. Therefore, the information available remains also limited due to the lack of randomized controlled trials[58-61]. The findings of these studies are inconsistent and may reflect different analytical approaches or selection bias. However, in biological studies, the effectiveness of treatment for toxoplasma infection has been reported to depend partly on the timing of treatment after infection[2]. The tachyzoite form of the parasite, which causes inflammation and necrosis, is highly sensitive to antibiotics, but it rapidly transforms into the latent encysted bradyzoite form, which is impenetrable to antibiotics[61]. Despite a lack of information on how fetal immune responses initiate and sustain cyst formation, if free tachyzoites persist in the fetus, there may be a prolonged period when prenatal treatment could reduce parasite damage[34].

No definite difference in the risk of congenital infection with treatment (with spiramycin or pyrimethamine-sulfadiazine) or no treatment was found in a large prospective cohort trial of 1208 pregnant women in Europe with primary *T.gondii* infection[62]. However, in other uncontrolled studies, prenatal treatment with spiramycin or pyrimethamine-sulfadiazine has been proven beneficial. In one study involving 5288 susceptible pregnancies, the risk of congenital toxoplasmosis was four times greater in neonates born to untreated mothers compared to that for neonates born to treated mothers[63]. Another study of 88 pregnant women with primary toxoplasmosis infection treated with spiramycin alone found a 0% rate of congenital toxoplasmosis at 2 years[64]. Thus, despite a lack of randomized studies, all pregnant women who have been diagnosed with primary toxoplasmosis infection should be treated[3].

The type of drug used may also be important. The combination of pyrimethamine, (adult dosage 25–100 mg/d×3–4 weeks), sulfadiazine adult dosage 1–1.5 g qid×3–4 weeks) and folinic acid (leucovorin, 10–25 mg with each dose of pyrimethamine, to avoid bone marrow suppression) is the basic treatment protocol[1]. In some cases, other drugs such as spiramycin (adult dosage 3–4 g/d×3–4 weeks) and sometimes clindamycin are recommended. Spiramycin is widely used to prevent placental infection in many European countries especially France, Asia and South America, while spiramycin prophylaxis is followed by a 4-week course of pyrimethamine plus sulfadiazine at 17 weeks of gestation in Austria and Germany. This approach has reduced the rate of clinical signs in the fetus[7]. In the US, although currently not approved by the FDA, spiramycin is available as an investigational drug upon special approval. Due to concerns regarding teratogenicity, pyrimethamine and sulfadiazine treatment for the prevention of fetal infection is contraindicated during the first trimester of pregnancy, except when the mother's health is seriously endangered. However, during the first trimester sulfadiazine can be used alone[1].

Antitoxoplasma treatment should be continued throughout pregnancy, and at least monthly ultrasound should be performed if the initial examination revealed no abnormalities; the presence of hydrocephalus has been used as an indication for termination of the pregnancy[7].

Treatment of congenital toxoplasmosis

Pyrimethamine and sulfadiazine, generally used to treat infants with congenital toxoplasmosis, have proven to improve outcomes of the infants treated with these drugs compared with untreated infants and children from studies in the past[2,32]. Drug therapy is usually continued for one year. Active and recurrent toxoplasmic eye disease

also frequently responds to antiparasitic drugs, which may be given with steroids[23].

Treatment is difficult to evaluate because of variations in severity and outcome of the infection and the disease. Treatment of disease (in contrast with infection) in humans apparently depends on the strain of parasite involved, the organs infected, and the time during the course of infection when treatment is initiated. However, the parasite is probably never completely eliminated by specific therapy, which can be recommended but is only beneficial against the tachyzoite form. None has been shown to effectively eradicate the encysted form, especially from the CNS and eye[2].

Proper evaluation of treatment in the asymptomatic infected infant is not possible because of insufficient data. Nevertheless, for most investigators, treatment for such infants should be undertaken in the hope of preventing the remarkably high incidence of late untoward sequelae seen in children who receive inadequate or no treatment[2,65].

Prevention

Prevention of Infection in Pregnant Womeni General recommendations for prevention of congenital toxoplasmosis in pregnant women start in the kitchen by cooking food to safe temperatures (71.1ºC or 160ºF) and using a kitchen thermometer to avoid undercooking. Freshly eaten fruits and vegetables should always be washed thoroughly and peeled whenever possible. Another important measure of preventing infection with *T.gondii* is using hot water and soap to wash utensils including the cutting board, dishes as well as the counters and hands following contact with raw meat, poultry, seafood, and with unwashed fruits or vegetables. Prevention of infection with *T.gondii* also starts by educating women of childbearing age about preventing *T. gondii* transmission from food and soil. For pregnant women, this prevention measure covers wearing gloves when gardening, working with soil or sand. Pregnant women should particularly try to avoid changing cat litter pans, cat feces being a major source of toxoplasma infection. However, if changing the cat litter box cannot be avoided, then, wearing gloves during this task as well as washing hands thoroughly with soap and hot water should be the common practice for pregnant women. Since the infectious cycle of *T.gondii* oocysts requires more than a day, changing the litter box on a daily base is a basic prevention approach. While avoiding interaction with cats other then their own, and keeping the house cat in doors is a common practice, feeding the cat with well-cooked or canned food contributes to preventing infection with *T.gondii*[66] . In summary, educating pregnant women at the first prenatal visit about food hygiene and avoiding exposure to cat feces and interaction with soil with bare hands is of prime importance. Two vital factors to be raised by the healthcare worker during this education should cover problems associated with *T.gondii* serology tests; it should be clearly stated that there is no assay that can determine precisely when initial T.gondii infection has occurred; secondly, a positive IgM result does not mean infection particularly in low incidence populations like the U.S. Although the incidence of toxoplasmosis is low in the U.S. population, it is imperative that the government and meat industry continue efforts to reduce the presence of *T.gondii* in meat[23]. In Turkey, Çetin et al found that the congenital *T.gondii* infection rate was 1.85% in random deliveries and 2.66% in the sick newborn population of theneonatal unit in their hospital[67]. These results were higher than in the U.S. and most of the European countries. Screening pregnant women is very important in countries such as Turkey where the incidence of congenital T.gondii infection is relatively high. *Prevention of Infection in Fetuses;* The first measure of prevention of infection in fetuses is the identification of the woman at risk by serologic testing. This would provide the opportunity to treat the patient during pregnancy, providing 60% reduction in infection among infants. However, if the patient has acquired the infection in the first or second trimester (<50% of cases), then therapeutic abortion is advised[2].

Screening In perinatal viral and parasitic infections the American College of Obstetricians and Gynecologists recommends toxoplasmosis screening only in high-risk persons or those in whom routine ultrasound examination (or ultrasonography performed for other reasons) presents findings such as hydrocephalus, intracranial calcifications, microcephaly, fetal growth retardation, ascites, or hepatosplenomegaly[68].

In addition, toxoplasmosis screening has been a routine program for pregnant women in France, in Austria and in the State of Goias, Brazil as recommended by experts[1]. Screening of women should begin prior to conception with follow-up monthly tests during pregnancy to detect seroconverion, which is the basis for the French screening program and the Austrian Toxoplasmosis Prevention Programs, recommending routine serologic testing. In Austria, it is recommended three times during pregnancy: in the first, second and third trimesters and in France six times following the initial finding[69,70]. When one of the tests suggests definite or probable primary maternal infection, treatment is recommended[71].

In Massachusetts, USA, where there is low seroprevalence in the population, only newborns are screened for the presence of *T. gondii*-specific IgM[72] followed by an extensive clinical evaluation and a one-year treatment regimen with combination of pyrimethamine and sulfadiazine[71]. A recent study screened 364,130 neonates in the United States for *T. gondii* specific IgM and confirmed 195 cases of congenital toxoplasmosis (1 in 1867). Moreover, no symptoms or at least no progress of the disease was determined during a 7-year follow-up of the treated patients. Based on these findings, the authors suggest including toxoplasmosis in neonatal screening programs[73].

Cost-effectiveness of optional screening programs (no screening, pre-conception or neonates screening, frequency of tests during pregnancy) depends on local factors: incidence of congenital toxoplasmosis, available diagnostic and therapeutic services, and the population compliance with screening. Promoting public, as well as professional, knowledge on the disease is essential in order to effectively prevent, diagnose and treat congenital toxoplasmosis[1].

In conclusion, screening programs of women at childbearing age and upon gestation or at least newborn screening is highly effective for early treatment and prevention of sequelae.

Vaccine

An effective vaccine against human *T gondii* infection is a desirable but difficult target. Only the attenuated live S48 strain of the parasite has been licensed for use in sheep in Europe and New Zealand[74]. Vaccine candidates that can induce protective Th1 and humoral (including IgA) responses—both systemic and at the intestinal mucosa level—have been the focus of recent research hoping to mimic the lifelong immunity conferred by natural infection. Some of the approaches to vaccine have included use of purified or recombinant *T gondii* surface antigens, live attenuated or mutant strains of the parasite, or DNA with plasmids encoding colony-stimulating factors[75-77].

Follow-up

Although sequelae in infants who are congenitally infected are most often ocular (e.g., chorioretinitis occurring at school age or adolescence), in some cases they are neurologic- for instance, convulsions may lead to discovery of cerebral calcifications or retinal scars. Ocular lesions may recur during childhood, adolescence, or adulthood. Neurologic relapses (e.g., late obstruction of the aqueduct) have also been reported[2].

Wallon et al reported the clinical evolution of ocular lesions and final visual function, in a prospective cohort of 327 congenitally infected children in France, who were identified by maternal prenatal screening and monitored for up to 14 years[78]. After 6 years, 79 (24%) children had at least one retinochoroidal lesion. In 23 children, a new lesion was diagnosed within10 years, mainly in a previously healthy location. Normal vision was found in about two thirds of

children with lesions in one eye, half the children with lesions in both eyes and none had bilateral visual impairment. However, most of the mothers (84%) had been treated. A combination of pyrimethamine and sulfadiazine had been prescribed in all the children (38% before and 72% after birth).

Clinicians, parents, and elder children with congenital infection should be informed that late-onset retinal lesions and relapse can occur many years after birth but that the overall ocular prognosis of congenital toxoplasmosis is satisfactory when infection is identified early and treated accordingly[78].

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