REVIEW ARTICLE

APPROACH TO CONGENITAL INTRAUTERINE INFECTIONS

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ABSTRACT: TORCH infections remain as one of the significant causes of recurrent pregnancy losses even today. Laboratory diagnosis like sero diagnosis, molecular methods, antigen detection, virus isolation, inclusion bodies play the key role in diagnosing and treating congenital intrauterine infections. Recommending prenatal diagnosis with provisional diagnosis of TORCH infections is important. When infection is suspected during pregnancy, to reduce the risk of fetal loss cordocentesis, amniocentesis should be recommended. Prenatal diagnosis before 8th week and after 34 gestational weeks is not recommended because of risk of fetal loss and risk of secondary infection.

KEYWORDS: Congenital infections, TORCH, IgG

INTRODUCTION: Pregnancy is always challenged with determination of pregnancy loss. This challenge is maximum in early pregnancy. One of the main reasons for abortion is intrauterine infections. All infections during pregnancy do not lead to intrauterine or fetal infection because placenta is an efficient barrier to prevent infections. Infections to fetus also can be from genital tract either ascending or descending or membrane rupture.

A large variety of organisms such as Chlamydia trachomatis, malaria, Leishmania donovani, Hepatitis B, and Hepatitis C may infect the pregnant women in the first few weeks of pregnancy, but these may not cause significant damage to the fetus.[1,2] Common organisms responsible for clinical manifestations in the fetus include Rubella virus, CMV, Toxoplasma gondii, Treponema pallidum, enterovirus such as Parvo-B19, measles, mumps, HN, and Varicella virus, etc.

The Toxoplasma gondii, Rubella, Cytomegalovirus, Herpes simplex virus and Syphilis have common clinical features, thus these organisms are also grouped as TORCH group of infections.

COUNSELING FOR TORCH INFECTIONS:

1. A detailed history along with detailed pelvic examination to rule out coexisting infections.
2. Hematocrit, platelet, and reticulocyte counts.
3. Culture and sensitivity of throat swab, urine, stool, cerebrospinal fluid (microcephaly, anancephaly, hydrocephaly), and vesicular fluid (Herpes simplex virus).
4. Antigen detection tests in cases of Cytomegalovirus infection.
5. Total IgM determination-(over 25 mg/100 ml suggests active viral disease).
6. Agent specific IgM/IgA antibodies;
7. X-ray films of long bones (to rule out rarefaction) and skull (intracranial calcifications).
8. Electrocardiogram for evidence of myocarditis (coxsackie B enterovirus).
9. Ophthalmic examination for evidence of chorioretinitis or cataract.

It is also important to know that Toxoplasma gondii and Rubella infections do not cause repeated abortions, a big misconception. These two infections, on the contrary give good protection for subsequent pregnancies. Several laboratories also report acute mixed infection of two or more TORCH agents which is not a common phenomenon and if at all, it should definitely not be reported until it is verified by a reference laboratory. However, many kits can have cross reactive antibodies to CMV and HSV, hence not specific.

TORCH INFECTIONS: TOXOPLASMOSIS: It is a protozoa Toxoplasma gondii, which is transmitted to humans through the ingestion of food or water contaminated with cat faeces or by eating under cooked meat of the infected swine, sheep or goat. The parasite can gets transmitted through placenta from the infected mother to her fetus. According to recent studies, its prevalence is more common in socio-economically backward, tribal and iodine deficient populations. In these populations the prevalence (IgG antibodies) of Toxoplasma gondii in adults varied from 40-75%. While in Delhi and surrounding areas its prevalence has been between 25-28% in last 10 years.[2-5]

Only 30 to 40% of these infected mothers would have infected their fetuses. Transmission of infection is reported to increase with gestation, i.e. later the infection in pregnancy more chances of transplacental transmission. But the damage to the fetus is indirectly proportional to the age of gestation, i.e. maximum damage during the first trimester. Identification of positive titers of IgM and IgG antibodies during pregnancy in women with previous negative titers of anti-Toxoplasma IgG antibodies suggests a proliferative (active) disease, which is dangerous to the fetus.[6,7]

SERODIAGNOSIS[1-8]:

1. IgM (μ chain-specific) antibody capture ELISA: This is the most commonly used method of diagnosing acute toxoplasmosis in an immunocompetent pregnant woman to rule out congenital infection. However, in neonates its absence does not necessarily exclude the congenital infection, since in many fetuses if the infection occurs very early, the IgM response may not appear. But its presence is almost certainly diagnostic (provided good quality test kits and standard test methods are used). For antenatal diagnosis the IgM and IgA antibodies can be detected in Amniotic fluid, and fetal blood. In AIDS patients most (>90%) of the Toxoplasma cases are due to reactivation and hence IgM antibodies are not demonstrated, as a result of B-cell anergy.
2. IgA specific ELISA.
3. IgM fluorescent antibody test. This is also based on the above principle but it requires a fluorescent microscope and not many laboratories have this facility.
4. Immunosorbent agglutination assay for IgM antibodies (ISAGA-IgM) test as the alternative gold standard, where Feldman Dye test cannot be performed.
5. Direct agglutination test. This is the most sensitive and specific test for diagnosing acute as well as chronic toxoplasmosis. For the diagnosis of acute /congenital toxoplasmosis acetone fixed (AC) antigen of T. gondii is used.

Test	Sensitivity	Specificity
IgG ELISA	90-100%	75-99%
FA	90-95%	90-95%
HS-DAT	95-100%	90-99%
IHA	70-90%	60-75%
Feldman Dye Test*	90-100%	99-100%

Table 1: Sensitivity and specificity of various serological tests

* The test is not routinely done due to associated risk of laboratory acquired Toxoplasma infections. This test is done only in reference laboratories as a Gold standard and most specific test to evaluate the sensitivity of other tests.

MOLECULAR METHODS[1,6,7]**:** Prenatal diagnostic techniques were previously based upon demonstration of specific and non-specific signs of fetal infection with T. gondii. It includes amniocentesis along with cordocentesis for fetal blood sampling. Amniocentesis could isolate T. gondii by mouse inoculation and cell culture, whereas both specific and non-specific tests were performed on fetal blood.

Studies of Grover et al[9] first reported on the accuracy and usefulness of PCR for direct detection of T. gondii DNA in amniotic fluid. In this study, PCR targeting the repeated B1 sequence gene of T. gondii was more sensitive than mouse inoculation of tissue culture and detected T. gondii in 8 to 10 AF samples of cases with proven congenital infection. Other promising results were obtained with PCR detection of several DNA targets of T. gondii, including the single copy gene of surface protein P30 or a one-hundred fold repeated sequence of 18s ribosomal DNA. These PCR techniques are simple to perform once standardized and are highly useful in prenatal diagnosis of intra-uterine Toxoplasmosis. The amniotic fluid is the best clinical sample, as fetal blood sampling carries a high risk of fetal loss than amniocentesis, the later is no more necessary.[7]

Specificity of the PCR methods using Bl gene target is about 94 to 97%. Significant variations in sensitivity have been demonstrated by Pelloux et al[10] using artificial AF samples spiked with tachyzoites of T.gondii. Among 15 European laboratories, involved in prenatal diagnosis, 9 were able to detect a single parasite whereas 2 found no Toxoplasma in any of the 8 positive aliquots. Again, no clear link with specific technical procedures was found to explain such important discrepancies. In a large screening program involving more than 35,000 pregnant women in Norway, Jenum et al[11] performed prenatal diagnosis with a nested PCR in 6 women. The nested PCR method used in this study detected only 2/6 fetal infections (sensitivity = 33%) as compared with other conventional methods. This rather low sensitivity may be explained with other PCR technique used but also by the fact that amniocentesis was performed as soon as maternal infection and amniocentesis. Moreover, contrasting with current recommendations, amniocentesis was performed in 12 weeks' pregnancy in some cases, although reliability of PCR before 18 weeks' pregnancy has not been evaluated. Thus, in order to avoid false negative results due to the delay of parasite transfer from placenta to the fetus, it is recommended to

perform prenatal diagnosis from 18 weeks pregnancy and at least 4 weeks after the estimated date of maternal infection.

PARASITE ISOLATION: From 20 weeks' pregnancy, 1.5 to 4 ml of fetal blood (FB) and 10 to 30 ml of amniotic fluid (AF) are sampled for analysis. Pellet of amniotic fluid and whole blood clot are injected intraperiotoneally or subcutaneously into mice. After 3 to 6 weeks, inoculated mice that develop a specific anti-Toxoplasma antibody response, are sacrificed and examined for the presence of brain cysts. Sensitivity of isolation of T. gondii either from fetal blood or amniotic fluid are similar and range from 49 to 72%. Isolation of T. gondii from tissue culture of amniotic fluid subsequently developed and allowed to obtain more rapid results within 4 days. However, this method revealed frequently less sensitive than mouse inoculation.[6,7]

ANTIGEN DETECTION: Antigen detection from the clinical samples such as amniotic fluid, urine, saliva, CSF, etc. can be done. But there are no commercially available diagnostic kits.

RUBELLA INFECTION: Rubella is caused by RNA virus of togaviridae family and genus Rubivirus. This is highly infectious but mild in nature, if acquired postnatally and spreads mainly through fomites. Seroprevalence of anti-Rubella IgG antibodies in females of child bearing age in India is reported between 70-90%, depending on the time of surveillance.[3,12] Due to the MMR vaccination, the population of immunity has increased significantly. Only those mothers who are IgG negative before pregnancy are vulnerable and can get freshly infected with Rubella, as the IgG antibodies provide excellent protection. However, approximately 50 to 60% of fetuses of women who contract rubella during the first month of pregnancy will be adversely affected by the virus. When the pregnant woman contracts rubella infection during the first trimester, incidence of malformation in the infant is about 35%; 25% when in the second month, and 10% when in the third month. After the fourth month, abnormalities, except for hearing defects, are uncommon.

The risk of congenital defects decreases with the advancement in gestation period reaching 10-15% in 4^{th} month and negligible in third trimester. Whether infants born to women who contract rubella during early pregnancy are obviously defective or not, but perinatal mortality is certainly high, and a high proportion of infants are of low birth weight in the past. Fetal damage can occur without obvious illness in the mother. The 1965 rubella epidemic in Europe involved many thousands of nonimmune pregnant women and resulted in damage to an extremely large proportion of their offspring during early fetal development.[12]

The Rubella virus readily invades the placenta and the fetus during gestation. Viremia during embryogenesis, and perhaps later in pregnancy, may produce signs and symptoms of persistent infection at birth. Appropriate cultures at this time for detection of the Rubella virus can prove fetal invasion with or without clinical evidence of disease. Excretion of the virus may persist throughout the first year despite measurable antibody titers.

This continued excretion creates a danger to non-immunized neonato-logists, gynecologists, nursing personnel and babies.

SERODIAGNOSIS: IgM (μ-chain specific) antibody ELISA test is highly specific and carries 99% positive predictive value for acute infection. Some women in spite of good health may deliver a healthy uninfected baby. Therefore, it is mandatory to examine the amniotic fluid/fetal blood for IgM antibodies before deciding for termination of pregnancy. 3% cases the IgM antibody against Rubella (natural infection and post vaccine both) may persist for years. Hence, one has to be very careful to rule out post vaccine persistence of these antibodies before advising drastic steps. It is also advisable that the IgM positive results must be re-confirmed by another test kit or by another laboratory.[11,12]

MOLECULAR METHODS: The detection of rubella-specific immunoglobulin M in fetal blood obtained by cordocentesis has been employed after 22 weeks of gestation, and even then false-negative results may be obtained. Nested RT- PCR based diagnosis is awardable on amniotic fluid, urine, fetal blood or mother's blood.[13]

Using these primers (first round R2 – 5'CAA CAC GCC GCA CGG ACA AC 3' – 8807-8826, R7 4'CCA CAA GCC GCG AGC AGT CA 3' – 8991-8972. Nested R11 – 5' CTC GAG GTC CAG GTC CYG CC 3' 8826-8845. R8C – 5' GAA TGG CGT TGG CAA ACC GG 3' 8968-8949), authors[13] have demonstrated that the method was highly specific amplifying all the nine strains of rubella virus (including 4 vaccine strains). No false positive PCR products were generated from the RNAs of 16 other RNA viruses or RNAs from 7 cell types or human DNA. The PCR is also reported to be highly sensitive and it has the potential to detect the Rubella RNA quantities of 10^{-6} equivalent to 0.1 $TCID_{50}$. The viral RNA can be amplified from various clinical fetal samples such as amniotic fluid, fetal lung, fetal kidney, fetal internal ear, fetal heart, fetal brain and other fetal tissues, placenta, chorionic villus tissues and other parts of conception.[13]

VIRUS ISOLATION: Isolation of rubella virus by cell culture from samples such as chorionic villi or amniotic fluid is possible, but rubella virus isolation is time-consuming.

CYTOMEGALOVIRUS INFECTION: It is caused by a (ds) DNA virus of herpes group. It is the commonest congenital infections. It is also the most common viral cause of mental retardation in children. It is reported to occur in 1-2% of all pregnancies in the United States. The infection is more common in low socio-economic groups. In India about 95% women of child bearing age are already infected with this virus. The common modes of infection are through saliva (kissing), urine, stool, breast milk and of course through unscreened blood transfusion. Toddlers in nurseries are richest source of the virus. The infection in majority of cases remains unnoticed. However, in contrast to other infectious agents, such as Rubella and Toxoplasma, this virus can infect the fetus despite maternal immunity, although maternal immunity seems to lessen the seventy of infection in fetus. Primary maternal infection with CMV carries a 50% risk of vertical transmission to the offspring with a greater chance of symptoms at birth and long-term handicap of the child.[1,14,15]

Sarman Singh[16], at All India Institute of Medical Sciences, New Delhi has done a prospective study, in which 930 pediatric patients with suspected congenital CMV infection (age 0 day-14 years) were included. Out of 930 serum samples, 188 (20.2%) were found positive for

CMV specific IgM antibodies using μ-chain specific EIA. Most of the infants (4.9%) had neonatal hepatitis (81%) neonatal cholestasis as a single manifestation. Of the toddlers and pediatric patients 15% had hepatomegaly. Of these 6.4% had jaundice while 8.6% had non-icteric hepatomegaly. Microcephaly was chief manifestation in 9.02% while hydrocephaly in 2.7% patients. Congenital cataract was detected in 11.2% cases while other ophthalmic deformities such as microphthalmos, uveitis, etc. were seen only in 2.4% cases. Coagulation disorders and unexplained anemia was chief complaint in as many as 11.4% of the cases. In more than one-third (34.8%) cases clinical manifestations were complex and suggestive of TORCH syndrome.[16]

SERODIAGNOSIS: IgM (μ-capture) ELISA test or Direct Immunofluorescence (IgM) Assay that use monoclonal antibodies raised against specific pp65 or other early stage-specific antigens. Such tests detect specific antibodies raised against the antigens expressed in the early stage of CMV infection. The IgG avidity can help in deciding the course of management.

MOLECULAR METHODS: The prevalence of IgG antibodies in India, in the women of child bearing age is 95%. The CMV remains latent for several years and even for the whole life but can also get reactivated even if there are high titers of IgG antibodies demonstrable in the blood. IgM antibodies do not rise to detectable levels. This results in a low negative predictive value of the IgM antibodies. Hence, molecular methods are definitely more sensitive and specific. The common molecular techniques in use are: PCR, n-PCR, RT-PCR, and Nucleic Acid Sequence-based Amplification (NASBA) or Nucleisense.[14,15]

The PCR can predict CMV disease 2-3 weeks in advance, the disadvantages of PCR include high rate of contamination, i.e. false positivity, high degree of skills needed and quantitation of the results/viral load is difficult.

RT-PCR- is cumbersome and involves several steps including reverse transcription. Has low sensitivity due to the use of spliced mRNA as target. NASBA uses un spliced mRNA leading to highest possible sensitivity and specificity. The NASBA is, isothermic and single tube test with chemiluminiscent detection system, but it is extremely costly. Therefore, the nested PCR remains the only reasonably sensitive, specific and affordable by most of the patients.

ANTIGEN DETECTION: pp65 antigen is a late and extracellular antigen, therefore, it is opsonized by peripheral blood lymphocytes. The pp65 antigen detection in amniotic fluid confirms the diagnosis of intrauterine CMV infection.

INCLUSION BODIES: Intracellular perinuclear inclusion bodies - an”owl's-eye" halo appearance in the shed epithelial cell in the urine concentrates stained with hematoxylin-eosin, can be seen in saliva and spinal fluid.

HERPES SIMPLEX VIRUS INFECTION: It is DNA virus of the herpes group. There are two serotypes which infect immunocompetent as well as immune compromised humans, commonly. However, human herpes virus (HHV)- 6 and HHV-8 are more common in AIDS patients. Herpes virus infection in the neonate is commonly acquired by contact with the mother's infected birth

canal (either HSV- type 1 or 2 but usually HSV-2). Though, occasional case reports of intrauterine malformations and congenital infection of the infant have been published, transplacental passage of the virus appears to be infrequent. Incubation period for herpes virus is apparently between 4 and 21 days.

Acquisition of the virus by the neonate in a genitally infected mother may be as high as 50%. HSV-2 has a predilection for the genital area, while HSV-1 has prediction to CNS.[1]

SERODIAGNOSIS: There will be elevated specific and total IgM, abnormal cerebrospinal fluid findings in herpes cephalitis (if CNS involvement), abnormal liver function studies and abnormal coagulation panel compatible with DIC. Enzyme-linked immunosorbent assay (ELISA) for (i-capture IgM antibodies can be used.

MOLECULAR METHODS: PCR primers have been introduced in the market but their usefulness particularly the specificity is not yet known.

INCLUSION BODIES: Characteristic multinucleated giant cell and intranuclear inclusions visible on a smear obtained from scrapings of vesicles, fixed with alcohol, and stained with Papanicolaou's stain.

ISOLATION OF VIRUS: Isolation of virus from vesicle fluid, throat washing, urine, blood, or cerebrospinal fluid, amniotic fluid or cervical swab placed in Leibowit-Emory transport medium. Typical cytopathic changes are visible on tissue culture within 2 to 9 days.

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