LABORATORY TESTS FOR DIAGNOSIS OF TORCH INFECTIONS

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Ever since the 1971 publication of CDC study of congenital infections, the acronym 'TORCH' has come into use(1). Etiological agents of various important infections which can be contracted *in-utero* were conveniently grouped together as TORCH where T stands for Toxoplasmosis, R for Rubella, C for Cytomegalovirus and H for Herpes. Some people have altered this mnemonic to include syphilis (TORCHS) whereas others have added 'O' for other agents causing similar disease in infants(2). Now there is a suggestion to add another H for human immunodeficiency virus and make it TORCHH(3).

Most of the TORCH agents produce common clinical features reflecting the multi organ and CNS involvement, hence this is termed as TORCH syndrome. "TORCH syndrome"(4) describes those children having congenital infection by one of the several organisms which are clinically indistinguishable. They are distinguished primarily by serologic or microbiologic investigations. The laboratory diagnosis of each one of them is briefly discussed below.

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Rubella Virus

Congenital Rubella

Primary rubella infection in a pregnant woman is accompanied by maternal viremia and this leads to transplacental transmission of the virus *in-utero* leading to congenital infection(5). The second attack of rubella can occur inspite of humoral immunity but is not associated with viremia and hence the products of conception are not affected(6).

When primary rubella occurs during pregnancy there are several possible outcomes. The fetus may escape infection completely, may be infected inapparently with subsequent pathologic consequences in later life or there may be damage to a single or multiple organs and tissues. Both apparent and inapparent primary infections in mother may result in transmission to fetus. The risk of congenital rubella is highest in first trimester of pregnancy(7) but *in-utero* transmission has been reported upto 5th month of pregnancy(8).

In congential infection acquired during late pregnancy, the child may be born apparently normal but late manifestations in the form of poor physical growth and developmental retardation, may be noted after a few years(9).

Congenital rubella infection is a prob-

lem in India(10). Based on detection of rubella specific IgM antibodies in the serum of symptomatic newborns and infants. We found that the incidence of congenital rubella infection was 14%(11).

Laboratory Diagnosis

In Pregnancy

(*i*) *Serology:* IgG antibodies persist throughout life after primary infection and are protective in nature. These antibodies can be detected by different tests like hemagglutination inhibition(HAI), enzyme linked immunosorbent assay (ELISA) and single radial hemolysis (SRH).

If a single serum sample is available, the IgM antibodies demonstration can help in diagnosing the primary infection but this should be done early in the disease as these class of antibodies last for 6-8 weeks in majority of people(12).

(*ii*) *Isolation:* This is the best method of to demonstrate the infection but cannot be used as a routine diagnostic procedure as it lacks the necessary sensitivity. Viremia is mainly present before onset of rash and hence isolation is most of the times not possible.

(*iii*) *Amniocentesis:* Amniocentesis can be used for *in-utero* diagnosis of the infection. Besides isolation of virus from amniotic fluid, detection of virus specific IgM antibodies in the fetus which can be done after 23-24 weeks of pregnancy by fetal blood sampling is also a promising test as is the detection of rubella virus genome and antigen in chorionic villi biopsy. These require further assessment(13).

In Infants

(*i*) *Serology:* The diagnosis of congenital infections in the newborn can be established by demonstrating virus specific IgM antibodies in the cord blood. IgM antibodies persist for a long time in these children due to persistence of virus in the body(14).

The demonstration of IgG antibodies can help in diagnosis of congenital infection if the blood samples of both mother and neonate are put up in the same set and then repeated after 3-6 months. The titres of matenal antibodies would remain essentially the same as before or would decline whereas a rise in the antibody titres is suggestive of congenital infection. The transplacentally acquired IgG antibodies have a half life of about one month, and the titres of passively acquired antibodies start declining in the neonate after 1-3 months. Serology after 1 year of age is not helpful because then it becomes difficult to say whether the antibodies are due to the congenital or community acquired infection.

(*ii*) *Isolation:* The virus can be isolated from most of the body fluids. The virus is present in 90% of the newborns in all the fluids at birth, 50% excrete virus upto 6 months of age and another 1% upto one year of age(12). There is a report of virus isolation from cataractous material at the age of 3 years(15).

Cytomegalo Virus (CMV)

Epidemiology

CMV is a ubiquitous agent. Congenital CMV infection occurs in approximately 1% of all livebirths. During the first year of life, additional large number of infants become infected as a result of either contact with infected genital secretion or breast milk(16). By the end of first year, about 40% of infants are excreting virus into the urine which is a source of infection to other children. In premature infants who are under intensive care unit, blood transfusion is

another important source of infection. The unique feature is the persistent or intermittent excretion of virus into saliva, urine, semen, cervical secretions and milk which helps in the transmission of virus to the close contacts(17). In India, different sero-epidemiologic studies have shown that by adulthood 80-100% of the normal population has antibodies to CMV(18).

Congenital CMV

CMV infection in pregnancy may be primary or recurrent but majority of these are recurrent(19). Intrauterine infection in early pregnancy may cause abortion or congenital defects and late in pregnancy, it may cause still birth, prematurity or a low birth weight child who may develop permanent disease in neonatal period or some sequalae in the later life(20).

Annual incidence of intra-uterine infection with CMV is 0.4-2.5% of all live births. Of the infected infants, 5-10% are symptomatic at birth; of the 90-95% infected asymptomatic, 5% develop late manifestations of CMV in early childhood(21). In our study, evidence of congenital CMV infection was seen in 19% of infants who were symptomatic(11).

Laboratory Diagnosis

In Pregnancy

The major problem during pregnancy is the difficulty of establishing the diagnosis of active infection in mother. Since majority of the infections are asymptomatic, the diagnosis rests on laboratory evidence.

(*i*) *Serology:* IgG antibodies persist throughout the life after primary infection. They can be detected by various tests like CFT, ELISA, IHA, IFA or RIA. IgM antibodies rise only during the primary infection.

(*ii*) *Isolation:* This remains the best method of diagnosis. Viral shedding persists for a few months after active infection. Virus isolation however is a tedious procedure and takes about 4-5 weeks.

(*iii*) *Amniocentesis:* Isolation of virus from amniotic fluid is a very good indicator of intrauterine infection. However, its role is still uncertain compared to the risk of the procedure.

Recently, some rapid methods have been found to be more useful in diagnosis:

(*a*) *Detection of Early CMV Antigen by IFA:* Early antigen of CMV has been detected in shell vial cultures using monoclonal antibodies by IFA. By this method virus can be detected in 24-48 hours(22).

(*b*) *Detection of Viral DNA by in-situ Hybridization:* This can be done by hybridizing the cervical scrappings with specific radio labelled probes of CMV(23).

(*c*) *Polymerase chain reaction (PCR):* This technique has been used to detect viral DNA in cervical smears and specific DNA sequence in urine samples using specific primer pairs. The DNA is amplified and then can be detected by specific radio-labelled probes(24).

In Infants

(*i*) *Serology:* Diagnosis of congenital CMV infection in a newborn can be done by demonstration of CMV specific IgM antibodies in the cord blood. The role of detection of IgG antibodies in the maternal and fetal blood samples is the same as discussed earlier in congenital Rubella infection.

(*ii*) *Isolation:* Viruria at birth is a direct evidence of congenital CMV infection and isolation of virus from the urine is a best method of diagnosis. Urine deposit of the infected child can be stained with Giemsa

and one can look for typical owl's eye intranuclear inclusions in the epithelial cells which are characteristics of CMV infection.

(*iii*) *PCR:* As discussed earlier, PCR can be done in the urine samples of the infected children.

The rapid tests are still under evaluation as far as their role in neonatal diagnosis is concerned.

Herpes Simplex Virus (HSV)

Congenital HSV Infection: Both HSV-2 and HSV-1 can be responsible for congenital infection but HSV-2 is a more common infection. Primary infection and reactivation of the latest virus can affect the fetus in pregnancy(12). Primary infections are worrisome because they imply the theoretical possibility of infection followed by viremia, whereas in secondary infection the presence of antibodies help in decreasing the severity of infection.

Congenital disease occurs more commonly by ascending infection prior to delivery or as a result of direct contact during delivery. The baby develops signs of infection with characteristic skin lesions 2 to 10 days after exposure. Ultimately systemic dissemination or CNS disease is produced in majority(25).

Laboratory Diagnosis

In Pregnancy

Diagnosis cannot be done on clinical grounds alone as most of the infections go unrecognized. Only where characteristic vesicular lesions are present, HSV infection can be suspected. Most of the times the diagnosis has to rest on laboratory evidence.

(*i*) *Virus Isolation:* Isolation from the vesicular fluid or cervical secretion is the optimal diagnostic approach. Cultures take 7-10 days to exclude the possibility of infection.

(*ii*) *Detection of antigen:* It can be done by ELISA or IFA from the patient's sample and helps in the rapid diagnosis(26).

(*iii*) *DNA probes:* Detection of HSV DNA in the cervical lesion using cloned Bgl IIN fragment as probes has been used for the diagnosis of herpes virus infection(27).

(*iv*) *PCR:* This has been used for diagnosis of herpetic encephalitis in CSF(28). Same can be possibly used for diagnosing the infection in cervical lesions.

(*v*) *Serology:* It is of limited value since most of the women of child bearing age are seroconverted before conception occurs and no single antibody titre is predictive of the presence or absence of genital shedding of virus at any point in time. Even if we take paired serum samples, the time required would invalidate the clinical value of this approach especially where rapid decision such as management during labor or following rupture of membranes has to be taken.

In Infants

(*i*) *Serology:* IgM antibodies detection can only help in diagnosing a congenitally acquired infection of the newborn. IgG antibodies and their role has already been discussed in rubella infection.

(*ii*) *Isolation:* Virus can be isolated from skin lesion or CSF of the infants in the similar way as in the mother.

Toxoplasma

Congenital Toxoplasmosis

The intrauterine transmission of

toxoplasma infection results in abortion, stillbirth or prematurity or it can cause congenitally infected symptomatic baby or asymptomtic child who develops illness later in life(29).

Incidence of congenital toxoplasmosis is 0.25-3% in different studies(30). Nearly, 70% of the infected infants show no signs and symptoms of infection in the early neonatal period. Brain damage is a common manifestation(31). In a significant number, the first sign of cerebral damage becomes evident after several years.

Earlier studies concluded that congenital infection can occur only when primary infection occurs in the mother but now it has been seen that even in the previously infected women, pregnancy can be complicated(30). In a study by Kimball *et al.*(32) out of 68% sero-negative women, only 6 sero converted during pregnancy and 2 transmitted infection whereas of the 1,283 seropositive at the time of pregnancy, 17 showed rise of titres in pregnancy of which in one there was transmission of infection to the fetus. This indicates that both primary and secondary infections can lead to congenital infections though the incidence is much lower in the secondary infections.

Laboratory Diagnosis

(*i*) *Serology:* The dye test, IHA, CFT and ELISA have been used but ELISA has been found to be more useful(33). Demonstration of IgM antibodies in neonate are helpful in establishing congenital infection. IgG antibodies are useful in case a paired serum sample is obtained as discussed earlier in other infections.

(*ii*) *Isolation:* This can be done by inoculating the sample in the peritoneal cavity of mouse. The limitations are that this is time consuming and laborious procedure.

Torch Evaluation and Torch Screening

TORCH agents produce a similar clinical picture which includes common clinical features like low birth weight, prematurity, purpura, jaundice, anemia, microcephaly/hydrocephaly, cerebral calcification, chorioretinitis, cataracts, microphthalmia and pneumonitis in a newborn(34). The use of this collective term for diagnosis and to direct investigations is both misleading and inappropriate(35). TORCH screening has been a useful shorthand for investigations of congenital infection but clinicians should be aware that the screen consists of a series of investigations on specific samples of which only some may be appropriate in a particular case. Sera often sent as a part of a TORCH screen are not helpful(35). Thus, recognition of one or more of the clinical signs of the TORCH syndrome can prompt the doctor to take appropriate samples, *e.g.,* (*i*) Cardiac lesions are mostly associated with rubella and CMV; (*ii*) Ocular lesions are very rare in herpes and cataract which is common in rubella seldom occurs with CMV and almost never in toxoplasmosis; (*iii*) Cerebral calcification should alert for toxoplasma infections; and (*iv*) deafness is almost always associated with CMV and rubella. In our study we found that the prevalence of cataract was significantly higher in congenital rubella and hepatosplenomegaly was significantly higher in congenital CMV infection(11).

The working group of PHLS was set to review diagnostic tests for congenital infections as many microbiologists are now questioning whether "Torch Screen" is appropriate since they feel that resources could be better spent if requests for laboratory procedures were more specific. The group recommended that the word "TORCH" should be abolished and

specific investigations should be asked for(36).

REFERENCES

1. Nahmias AJ, Walls KW, Stewart JA, Herrmann KL, Flynt WJ. The Torch Complex. Perinatal infections associated with Toxoplasma, Rubella, Cytomegalovirus and Herpes. Pediatr Res 1971, 5: 405-407.
2. Nahmias AJ. The TORCH complex. Hosp Pract 1974, 9: 65-66.
3. Sherman RA. The TORCH syndrome revisited. Pediatr Infect Dis J 1989; 8: 62.
4. Fine JA, Arndt KA. The TORCH syndorme: A clinical review. J Am Acad Dermatol 1985, 12: 697-701.
5. Haukewes G, Haran KO. Clinical rubella after reinfection. New Engl J Med 1973, 258: 525-526.
6. Cooper LZ, Krugman S. Clinical manifestations of postnatal and congenital rubella. Arch Opthalmol 1967, 77: 434-436.
7. Lorber J, Stevenson J, Hambling MH, *et al*. Diseases due to infection. *In:* Textbook of Pediatrics, 3rd edn. Eds Forfar JO, Arneil GC. Edinburgh, Churchill Livingstone, 1984, p 1424.
8. Vegtrop M, Mausa B. Rubella IgM antibodies in sera from infants both after maternal rubella later than 12 weeks of pregnancy. Scand J Infect Dis 1980, 12: 1-4.
9. White DO, Fenner F. Togaviruses and Flaviviruses. *In:* Medical Virology, 3rd edn. Chicago, Academic Press, Inc, 1986, p 499.
10. Manjunath N, Balaya S. Serological study on congenital rubella in Delhi. Indian J Med Res 1984, 79: 716-721.
11. Broor S, Kapil A, Kishore J, Seth P. Prevalence of cytomegalovirus and rubella virus infections in suspected cases of congenital malformations. Indian J Pediatr 1991, 58: 75-77.
12. Banatvala JE, Best JM. Rubella. *In:* Principles of Bacteriology, Virology and Immunity, 8th edn. Eds Collier LH, Timbury MC. London, Edward Arnold, 1990, pp 501-532.
13. Capner PM. Diagnosing rubella. Brit Med J 1989, 299: 338-339.
14. Al-Nakib W, Best JM, Banatvala JE. Rubella specific serum and nasopharynx immunoglobulin responses following naturally acquired and vaccine induced infection: Prolonged persistence of virus specific IgM. Lancet 1975, 1: 182-185.
15. Menser MA, Harley JD, Hertzberg R, Dorman DC, Murphy AM. Persistence of virus in lens for 3 years after perinatal Rubella. Lancet 1967, 2: 387.
16. Stagno S, Reynolds DW, Pass RF, Alford CA. Breast milk with risk of CMV. New Engl J Med 1980, 302: 1073-1076.
17. Stern H, Tucker SM. Prospective study of CMV infection in pregnancy. Brit Med J 1973, 2: 268-270.
18. Pal SR, Chitkara NL, Krech U. Seroepidemiology of cytomegalo virus infection in and around Chandigarh (Northern India). Indian J Med Res 1972, 60: 973-978.
19. Reynolds DW, Stagno SP, Hosty TS, Tiller M, Alford CA. Maternal CMV excretion and perinatal infection. New Engl J Med 1973, 289: 1-5.
20. Starr JG, Bart BD, Gold E. Inapparent congenital CMV infection: Clinical and epidemiologic characteristics in early infancy. N Engl J Med 1970, 282: 1075-1078.
21. Griffith PD, Campbell AB, Health RB. Prospective study of primary CMV infection in pregnant women. Brit J Obstet Gynecol 1980, 87: 208-314.

22. Leland DS, Nonsing RL, French MLV. Clinical experience with CMV isolation using conventional cell cultures and early antigen detection in centrifugation enhanced shell vial cultures. J Clin Microbiol 1989, 27: 1159-1162.

23. Gleaves CA, Hursh DA, Rice DH, Meyers JD. Detection of CMV from clinical specimens in centrifugation culture by *in-situ* hybridization and monoclonal antibody staining. J Clin Microbiol 1989, 27: 21-24.

24. Demmter GJ, Buffone GJ, Schimbor CM, May RA. Detection of CMV in urine from reaction DNA amplification. J Infect Dis 1988, 158: 1177.

25. Nahmias AJ, Dowdle WR, Josey WE, *et al*. Newborn infection with Herpes types 1 and 2. J Pediatr 1969, 75: 1194-1197.

26. Dascal A, Chan Thim J, Morahan M, Parnoy J, Mendelson J. Diagnosis of HSV infection in clinical setting by direct antigen detection enzyme immunoassay kit. J Clin Microbiol 1989, 27: 700-702.

27. Manjunath N, Kaur H, Bala S, Kaur R, Bhargava V, Rath GK, Seth P. Detection of HSV-2 DNA in uterine-cervix lesion using cloned BgL II N fragment of HSV-2 DNA as a probe. Indian J Med Res 1988, 87: 127-133.

28. Rowley AH, Whitley R, Lakeman FD, Wolinsky SM. Rapid detection of HSV DNA in cerebrospinal fluid of patients with herpes simplex encephalitis. Lancet 1990, 335: 440-441.

29. Feldman HA. Toxoplasmosis. New Eng J Med 1965, 279: 1370-1372.

30. Reminton JS. Toxoplasmosis. *In:* Obstetric and Perinatal Infections. Eds Charles D, Finland M. Philadelphia, Lea and Febringer, 1973.

31. Longer H. Repeated congenital infections with *Toxoplasma gondii*. Obstet Gyanecol 1963, 21: 318-320.

32. Kimball AC, Kean BI, Fuchs F. Congenital toxoplasmosis: A prospective study of 4,048 obstetric patients. Am J Obstet Gynecol 1971, 111: 211.

33. Beaver PC, Jung EC, Cupp EW. Clinical Parasitology. Philadelphia, Lea and Fabringer, 1984, pp 149-178.

34. Nahmias AJ, Josey WE, Naib ZM, Freeman MC, Fernandez RJ, Wheeler JH. Perinatal risk associated with maternal genital herpes simplex virus infection. Am J Obstet Gynecol 1971, 110: 825-827.

35. Editorial. TORCH syndrome and TORCH screening. Lancet 1990, 335: 1559-1561.

36. Public Health Laboratory Service—TORCH Screening Reassessed. London PHLS, 1990.

EMERGENCY TIPS

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Triaging Head Trauma

Closed head injury is one of the most common reasons for hospital admission. The workup of these patients remains controversial. Recent reports have questioned the reliability of plain skull *X*-rays? Most clinicians advocate CT scanning of head as the examination of choice in patients with minimal head injuries (MHI). Despite the fact that CT scanning allows the diagnosis of intracerebral injury to be

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