Serological Study For TORCH Infections In Women With High Delivery Risk Factors In Mosul

Anmar Ahmed Dawood AL – Taie
Dept. of Biology , College of Science , University of Mosul , Mosul , Iraq
(Received 2 / 6 / 2008 , Accepted 1 / 3 / 2009)

Abstract
Objective: To evaluate the incidence of TORCH infections in women having history of pregnancy loss (PL) and women with high delivery risk factors (HDRF) in Mosul / Iraq.
Setting : A prospective study conducted during period from July 2006 to June 2007 .
Methods : The study included (100) women with (HDRF) and (50) clinically normal women with previous normal pregnancy and full term deliveries . Serological evolution for TORCH infections was carried out by IgG and IgM ELISA method .
Results : The acronym TORCH (Toxoplasma, Other infections ,Rubella, Cytomegalovirus, Herpes simplex virus) was introduced to highlight a group of agents which cause congenital and perinatal infections. The prevalence of TORCH infections in Mosul are examined in this study.
Toxoplasmosis is with a high risk infection seropositivity rate of only 43% among women of child-bearing age , 12% of them also are already seropositive for cytomegalovirus (CMV) and therefore most cases of congenital CMV infection are likely to result from maternal reinfection.
Rubella infections still occur each year and it appear in 16% . Neonatal Herpes simplex virus (HSV) infection is also low in Mosul 11%. It is apparent that requests for TORCH screening has been over-ordered and clinicians should be encouraged to send appropriate specimens for specific tests depending on the clinical features of the individual case so as to reduce the adverse fetal outcome .
Keywords: TORCH , Seroprevalence , high delivery risk factors , Pregnancy loss .

Introduction
The TORCH test, which is sometimes called the TORCH panel, belongs to a category of blood tests called Infectious-Disease Antibody Titer tests IDAT(1).
TORCH tests measured the presence of antibodies against a specific group of infectious diseases and their level of concentration in the blood. TORCH, an acronym for measuring the levels of an antibodies against groups of chronic infections: Toxoplasmosis, Other infections, Rubella, Cytomegalovirus (CMV), and Herpes simplex virus (HSV), may be acquired by a woman during pregnancy with disastrous consequences for the infant. All are grouped together because they can cause a cluster of symptomatic birth defects in newborns, collectively called the TORCH syndrome (2).Rahway have suggested that this classification is too limiting and that several additional infectious agents should be considered in the Other category, such as enteroviruses, *Borrelia burgdorferi* (the cause of Lyme Disease), and, of course, human immunodeficiency virus HIV (3) .
A positive IgG antibody test is usually a sign of past-exposure to the TORCH agent and is not a marker for current active infection. Detection of IgM antibody is more difficult, and false negative and false positive results may occur (4).
Toxoplasmosis is caused by *Toxoplasma gondii*, and is found in human worldwide, a parasite that the mother can acquire from handling infected cats, drinking unpasteurized milk, or eating contaminated meat. The infection is carried to the infant through the mother's placenta, and can cause infections of the eyes or central nervous system. The later in pregnancy that the mother is infected, the higher the probability that the fetus will be infected. On the other hand, toxoplasmosis early in pregnancy is more likely to cause a miscarriage or serious birth defects.(2,5,6) .
Syphilis was added to the TORCH panel because of a rapid increase in reported cases since 1990 (6,7,8). It is also a potentially life-threatening infection for the fetus.
Rubella is a virus that has a seasonal pattern, with epidemics most likely in the spring. Between 0.1-2% of newborns will be infected with rubella. The rate of fetal infection varies according to the timing of the mother's infection during pregnancy. Birth defects, however, are most likely (85%) in infants infected during the first eight weeks of pregnancy(9).
Cytomegalovirus (CMV) belongs to the herpesvirus group of infections. It can be transmitted through body secretions, as well as by sexual contact; some newborns which acquire CMV through the mother's breast milk Infected infants may have severe problems, such as hearing loss, mental retardation, pneumonia, hepatitis, or blood disorders(10).
Herpes simplex virus the virus enters the infant through his eyes, skin, mouth, and upper respiratory tract. infants born with HSV infection, about 20% will have localized infections of the eyes, mouth, or skin, about 50% of infected infants will develop disease spread throughout the body (disseminated) within (9-11) days after birth (11,12).
HSV-2 is sexually transmitted. Symptoms include genital ulcers or sores. In addition to oral and genital sores, the virus can also lead to complications such as infection of the lining of the brain and the brain itself (meningoencephalitis) or infection of the eye especially the conjunctiva and cornea (13).
The aim of the present study is confirm the presence of IgM antibody for TORCH by ELISA method in women with high delivery risk factors.

Patients and Methods
A prospective study was done from July 2006 to June 2007, on patients who had attended to the private laboratories in Mosul city . A total of 150 women were

investigated including 100 with high delivery risk factors and 50 clinically normal women with previous normal pregnancy and full term deliveries. Cases were included in the study depending on previous history of having 2-3 pregnancy loss, intrauterine deaths, preterm deliveries and intrauterine growth retardation.

From each woman 3 ml of venous blood was collected in a container with strict aseptic precautions. The serum was used for serological evaluation for IgM antibodies for TORCH infections according to manufacturer's instructions using ELISA techniques (9 , 14) .

TORCH index of each determination was calculated by dividing the value of each sample by calibrator values and TORCH M index of 1.0 or greater was considered positive for antibodies. (15 ,16 , 17).

Results

From 100 cases with (HDRF) , abortion occur in (38%), intrauterine growth retardation in (٢٣%), intrauterine death in (13%), premature labor in (٦%), early neonatal death in (8%), and congenital malformation in (12%).

The highest number (57%) of (HDRF) cases were in the age group of 21-30 years , (23%) in the age group of 31-40 years , and 5 in the >41 years . (Table 1)

Out of 100 (HDRF) cases (94%) and out of the 50 healthy controls 12(24%) were serologically positive for only one of the TORCH infections.In (HDRF) cases the seropositivity for *T. gondii* was 43%, CMV 24% , rubella virus 16% and HSV 11% , while in the control cases the seropositivity for *T. gondii* 12%, CMV 6% rubella 4% and HSV was 2% . (Table 2) .

The highest seropositivity in cases of repeated abortions was seen with *T. gondii* (39.4%) . In intrauterine growth retardation, *T. gondii* showed highest seropositivity (52.2%) , followed by CMV (21.7%) and rubella (17.4%). In intrauterine death and preterm labor *T. gondii* showed highest seropositivity of (53.8%) and (66.7%) respectively. In early neonatal death cases, rubella showed (37.5%) and *T. gondii* and CMV showed seropositivity (25% in each). In congenital malformation seropositivity with rubella was predominant (33.3%) and *T. gondii* and CMV showed (25%). One case of mixed infection was found in those with history of abortions (Table 3).

Table 1. The Distribution of patients and control according to age groups

Age Years	Seropositivity HDRF (n= 100)		Seropositivity Controls (n= 50)	
	Number	%	Number	%
١٠–٢٠	١٥	١٥	٢٤	٤٨
٢١ – ٣٠	٥٧	٥٧	٧	١٤
٣١ – ٤٠	٢٣	٢٣	١٦	٣٢
> ٤١	٥	٥	٣	٦
Total	١٠٠	١٠٠	٥٠	١٠٠

Table 2. The seropositivity of each infections agent within TORCH tests

TORCH agents	Seropositivity HDRF (n= 100)		Seropositivity Controls (n= 50)	
	NO	Percent	NO.	Percent
Toxoplasma	43*	43	6	12
Rubella	16	16	2	4
Cytomegalovirus	24*	24	3	6
Herpes simplex virus	11	11	1	2
Total	94	94	12	24

* = Six cases showed mixed with toxoplasma and cytomegalovirus .

Table 3. TORCH agents with different presentation of HDRF cases.

high delivery risk factors		Toxoplasma* +ve		Rubella +ve		Cytomegalovirus* +ve		Herpes simplex virus +ve		Total
Types of Risk Factors	Number of Patients	Number	Percent	Number	Percent	Number	Percent	Number	Percent	
Abortion	٣٨	١٥	٣٩,٤	٣	٩ .٧	١٠	٢٦,٣	٨	٢١,١	٣٦
Intrauterine growth restriction	٢٣	١٢	٥٢,٢	٤	١٧,٤	٥	٢١,٧	١	٤,٣	٢٢
Intrauterine death	١٣	٧	٥٣,٨	٢	١٥,٣	٣	٢٣,١	١	٧,٧	١٣
Preterm labor	٦	٤	٦٦,٧	٠	٠	١	١٦,٧	٠	٠	٥
Early neonatal death	٨	٢	٢٥	٣	٣٧,٥	٢	٢٥	٠	٠	٧
Congenital malformation	١٢	٣	٢٥	٤	٣٣,٣	٣	٢٥	١	٨,٣	١١
Total	١٠٠	43*	43*	16	16	24*	24*	11	11	٩٤

* = Six cases showed mixed with toxoplasma and cytomegalovirus .

Discussion

TORCH screening is now widely requested by clinicians, investigating suspected cases of congenital and perinatal infection.There is concern that such requests are inappropriate should be targeted more specifically (5).

It is evident that maternal infections play a critical role in pregnancy loss and their occurrence in patients with HDRF is a significant factor. Persistence of encysted forms of *T. gondii* in chronically infected uteri, and their subsequent rupture during placentation lead to infection of the baby in the first trimester and often to recurrent miscarriages (6). In the present study *T. gondii*, which is a known etiological agent in recurrent pregnancy loss was found in 43% pregnant women with HDRF , this is similar to what has been reported earlier (14 ,18, 19) . Congenital transmission of *T. gondii* is known to occur during the acute phase of maternal infection and the IgM antibodies are evaluated in the maternal sera (20,21) . IgM antibodies were found in 39.4% of present cases with recurrent abortions compared with other study reported previously showed (66.3%) of women infected with *T.gondii* which is to accentuate my study (19).

Pregnant women should have their blood examined for Toxoplasma antibody and those with negative results should take measure to prevent infection by avoiding exposure to cat feces, cooking meat thoroughly, and washing hands thoroughly after handling raw meat (22).

TORCH infections are unique in their pathogenesis and have potentially devastating clinical manifestations. Congenital toxoplasmosis remains an important cause of blindness, although avoiding exposure to cats and uncooked meat can prevent it (21) .

Cytomegalovirus remains the most common cause of congenital infection in the United States, the possibility of effective treatment with Ganciclovir has emerged from recent studies done by Hoffman-LaRoche, Basel, Switzerland (20). In neonatal herpes, selective use of cesarean delivery and antiviral therapy can decrease incidence and improve outcomes (11 , 16 , 23).

Both CMV and HSV are known to have an intrauterine route of transmission with significant mortality and morbidity (21). The present study shows seropositivity rate of 24% for CMV specific IgM in women with HDRF.In other studies seropositivity ranges from 3 to 12.9% (11 , 12 , 20,21).

Primary CMV infection in pregnancy has a higher incidence of symptomatic congenital infection and fetal loss. This infection, being asymtomatic in adults it is difficult to diagnose clinically. Demonstration of IgM antibodies is indicative of primary infection.The need of serological evaluation of CMV specific IgM during pregnancy has been supported by various investigators (12 ,24) .

Patients with HIV disease should have toxoplasma antibody titers checked. If the results of the blood test are positive and if the CD4 count is less than 100, patients should be given prophylactic antibiotics (trimethoprim-sulfamethoxazole is the medication of choice) with antiretroviral therapy until the CD4 cell count has risen (22).

Primary infection with HSV II acquired by women during pregnancy accounts for half of the morbidity and mortality from HSV II among neonates, the other half results from reactivation of old infection. Seropositivity rate of HSV IgM among the HDRF patients in our study was 10% , while HSV in asymptomatic women with recurrent infection during pregnancy was found to be 2% previously (25).

Seropositivity rate for HSV IgM among HDRF patients in our study was 11%, similar to what has been reported in other study (26) .

Mixed infection were noted in 17 out of 40 patients (42.5%) in association with *Toxoplasma* IgM antibodies in our study. Out of 17 patients of mixed infection 13 were with *Rubella*, two with CMV, one with Rubella plus CMV and one with CMV plus HSVII, similar

observation of mixed infection has been made earlier (17).

Rubella is a mild viral illness in children but can occasionally infect adults. Primary virus infection during pregnancy may cause fetal damage. In our study seropositivity for rubella was 16% while other workers reported seropositivity ranging from 4 to 17.7% (11 , 12) .

Episodes of increased incidence of Rubella are reported to occur every 3-4 years (25), since 10-20% of women in child bearing age are susceptible to Rubella (4), increased incidence of Rubella will lead to increased reporting of pregnant women with Rubella infection , (26.8%) pregnant women were positive for Rubella IgM as has been reported earlier (27). The observation therefore suggests an increased incidence of Rubella infection in pregnant women.

The IgG antibody in the pregnant woman may be a sign of past infection with one of these infectious agents. By testing a second blood sample drawn two weeks later, the level of antibody can be compared. If the second blood draw shows an increase in IgG antibody, it may indicate a recent infection with the infectious agent (5 , 17) .

IgM is never zero as it cross-reacts with many other IgMs and other idea proteins. We have to follow the reference range provided by the private laboratories (13, 19).

There is no direct relation between an active infection and ultrasound growth and, therefore ultrasound not be relied upon as the diagnostic criteria either to confirm or refute the diagnosis of Rubella. In fact it gives us no information at all. You cannot take a decision based on the ultrasound report therefore IgM is more specific and reliable (4).

The unborn child cannot be tested for infection by ultrasound. If there is a reasonable suspicion of a fetal infection the only way to check would be to take the blood from the fetus at twenty weeks and analyze it for IgM against Rubella and do a Polymerase Chain Reaction (PCR) diagnosis of Rubella (13 , 28).

IgM is a specific class of antibodies that seeks out virus particles. It is, therefore, the most useful indicator of the presence of a TORCH infection(9). The general abnormal, or positive finding give high levels of IgM antibody (20). The test can be refined further for antibodies specific to given disease agents. The TORCH screen, however, can produce both false-positive and false-negative findings (12).

IgM antibodies against TORCH organisms usually persist for about three months, while IgG antibodies remain detectable for a lifetime, providing immunity and preventing or reducing the severity of reinfection (26) , thus, if IgM antibodies are present in a pregnant woman, a current or recent infection with the organism has occurred. If IgM antibodies are absent and IgG antibodies are present and do not demonstrate an increase on serial testing several weeks later, it can be assumed that the person has had a previous infection by the corresponding organism (24) , or has been vaccinated to prevent an infection. If the serum of a person has no evidence of either IgM or IgG antibodies specific for the organism, then the person is at risk of infection because they do not have any demonstrable immunity (7).

References

1. Jawetz E, Melnick JL, Adelberg EA, Brooks GF. Herpesviruses Chapter 33. *In*: Jawetz, Melnick and Adelberg medical microbiology. 23 rd ed. USA: Lange Medical Books/Mcgrawhill; (2004) 443:435 .
2. Gomella, T.L. Infectious Diseases: TORCH Infections. In Neonatology: Management, Procedures, On-Call Problems, Diseases and Drugs, Norwalk, CT: Appleton & Lange .(1994).
3. Rahway,R. B. Pediatrics and Genetics: Disturbances in Newborns and Infants." In The Merck Manual of Diagnosis and Therapy. 16th ed. NJ: Merck Research Laboratories. (1992).
4. Thapliyal, N. , Jain, G., and Pandey ,G. Torch Test Need for Use as a Screening Test,Indian J for practicing doctor , (2005) Vol 1 , No . 4 .
5. Levin , Myron J. "Infections: Viral & Rickettsial." In Current Pediatric Diagnosis & Treatment, edited by William W. Hay Jr., Stamford: Appleton & Lange, (1997) 132 .
6. Cruse, J. M., and Robert E. L. Illustrated Dictionary of Immunology. New York: CRC Press(2003).
7. Gomella ,T. L. Procedures: Heelstick (Capillary Blood Sampling). In Neonatology: Management, Procedures, On-Call Problems, Diseases and Drugs, Norwalk, CT: Appleton & Lange (1994).
8. Frey, R. JGale Encyclopedia of Medicine, Gale Group.(2002).
9. Newton, E Diagnosis of perinatal TORCH infections. Clin Obstet Gynecol; .(1999) 42:59-70.
10. Lewis, R. A. Torch screen , Columbia University Pediatric Faculty Practice, NY. Review provided by Veri Med Healthcare Network (2007) .
11. Kapil, S. and Broor, S. Primary cytomegalovirus infection in pregnant and nonpregnant women in India. Indian J Med Microbiol ; (1992) 10:53.
12. Frey, R. J. TORCH Tests. Gale Encyclopedia of Medicine. 1st Edition. Gale Research Group (1999).
13. Surpam, R.B., Kmlakar, U.P., Khadse, R.K., Qazi, M.S., Jalgaonkar, S.V. Serological study for TORCH infection in women with bad obstetric history. J Obstet Gynaecol India; (2006) 56:41-3.
14. Zagar, A., Wani, A. and Masoodi, S. Seroprevalence of toxoplasmosis in women with recurrent abortion and neonatal deaths, and its treatment outcome. Ind J Pathol Microbiol; (1999) 42:482-3
15. Yashodhara, P., Ramlaxmi, B.A., Naidu, A.N. and Raman, L. Prevalence of specific IgM due to Toxoplasma, Rubella, Cytomegalovirus and C. trachomoatis infection during pregnancy. Indian J Med Microbiol; (2001) 19:79-82.

16. Sharma, P., Gupta, T., Ganguly, N.K., Mahajan, R.C. and Malla, N. Increasing Toxoplasma seropositivity in women with bad obstetric history and in new borns. Natl Med J; (1997) 10:65-66.
17. Mookherjee, N., Gogate, A. and Shah,P.K. Microbiology evaluation of women with bad obstetric history. Indian J Med Res; (1995) 102:103-107.
18. Yelikar, K. and Bhat, S. Maternal toxoplasmosis in repeated pregnancy loss. J Obstet Gynecol India; (1996) 46:29-31.
19. Abdulla, B.A., Hassan,S.A. and Al-Khffaf , F.H. The use of latex agglutination test in the diagnosis of toxoplasmosis among women in child bearing age in nenavah governorate in 2002 ,Iraq .Rafidain Journal of Science , (2002) Vol(14), No.(3).
20. Sue, G., Boyer, MN. RN., Kenneth, M. and Boyer, M.D. TORCH Infections in the Newborn Infant , Department of Maternal–Child Health, College of Nursing, University of Illinois at Chicago, Chicago, IL, USA and Department of Pediatrics, Rush University Medical Center, Chicago, IL, USA , (2004) Vol 4, No5 .
21. Sood, S., Pillai, P. and Raghunath, C. Infection as a cause of spontaneous abortion with special reference to Toxoplasma gondii, rubella virus, CMV and Treponema pallidum. Ind J Med Microbiol; (1994) 12:204-7.
22. Gandhi, M. Division of Infectious Diseases, UCSF, San Francisco, CA. Review provided by VeriMed Healthcare Network (2006).
23. Malhotra, V., Bhardwaj, Y. Comparison of enzyme linked immunosorbant assay and indirect haemagglutination test in serological diagnosis of toxoplasmosis. J Communicable Dis; (1991) 23:154-6
24. Berkow, R. Pediatrics and Genetics: Disturbances in Newborns and Infants. In vol. II, Rahway, NJ: Merck Research Laboratories (1992).
25. Lim,W.L. Seroimmunity to measles, mumps, rubella and poliomyelitis in Hong Kong. Hong Kong J Paediatr; (1992) 1: 34-40.
26. Cruse, J. M. and Robert, E. L. *Illustrated Dictionary of Immunology*. New York: CRC Press (1995).
27. Fowler, K.B., Stagno, S., Pass, R.F., Britt, N.J., Boll, T.J. and Alford, C.A. The outcome of congenital cytomegalovirus infection to maternal antibody status. N Engl J Med ; (1992) 326: 663-7.
28. Kadri, M. Torch Test, Indian J for practicing doctor ; (2005) Vol 1 , No . 4 .

دراسة مصلية للاصابة بالـ TORCH في النساء اللواتي لديهن عوامل خطورة عالية في مدينة الموصل

انمار احمد داود الطائي

قسم علوم الحياة ، كلية العلوم ، جامعة الموصل ، الموصل ، العراق

(تاريخ الاستلام: ٢ / ٦ /٢٠٠٨ ، تاريخ القبول: ١ / ٣ / ٢٠٠٩)

الملخص

الموضوع: تقييم حالات الإصابات بالـ TORCH في حالات ضياع الحمل للنساء اللواتي لديهن عوامل خطورة عالية في الموصل / العراق .

تاريخ البحث : اجريت دراسة مستقبلية خلال المدة بين شهرتموز ٢٠٠٦ و شهر حزيران ٢٠٠٧ .

الطريقة : تضمنت الدراسة (١٠٠) امرأة ممن لديهن تاريخ ولادي (إنجابي) عالي الخطورة و (٥٠) امرأة طبيعية سريريا مع حالات ولادة طبيعية سابقة (مدة حمل كاملة).

التطور المصلي لحالات الاصابة بالـ TORCH أجريت بوساطة طريقة الاليزا للكلوبيولين المناعي المصلي IgG , IgM .

النتائج : يعني اصطلاح الـ TORCH (داء القط ، اصابات فايروسية اخرى ، الحصبة الالمانية ، فايروس تضخم الخلايا – العقبولة) ادخلت لتوضيح مجموعة من العوامل التي تسبب التشوهات الخلقية التي تحدث اثناء الحمل .

هناك اهتمام في ان هذه الفحوصات يجب ان تكون هادفة ودقيقة ، ان مسببات الاصابة بالـTORCH في الموصل هي التي سيتم التحري عنها في هذه الدراسة ، اذ ظهر داء القط له معدل عالي ايجابي للمصل بنسبة (٤٣%) ضمن النساء اللواتي بسن الانجاب ، و(١٢%) منهن ضمن عمرالانجاب ايضا ظهر لديهن ايجابية مصلية لفايروس تضخم الخلايا حيث ان معظم الاصابات كانت نتيجة لاعادة الاصابة للام الحامل .

اصابة الحصبة الالمانية لازالت تحدث وظهرت بنسبة (١٦%) اما اصابة العقبولة قليلة في الموصل ومع هذا ظهرت بنسبة (١١%) .

من الواضح ان التحري عن الـ TORCH قد تم الطلب عليه بشكل كبير ومن الضروري تشجيع الاطباء لارسال النماذج المناسبة للفحوصات الدقيقة المعتمدة على الاعراض السريرية للمرضى وذلك للتقليل من النتائج العكسية على الجنين .

المفتاح : TORCH ، تحري مصلي ، وجود عوامل خطورة ، فقدان الحمل اوضياع الحمل .