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ORIGINAL ARTICLE

Evaluation of immunity against CMV in Azarbaijan female population

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ABSTRACT

Background: Many viral infections are associated with significant maternal and fetal consequences if acquired during pregnancy. In this study we wanted to clarify or remind some of the failures of laboratory techniques or managements.
Patients and methods: In this study 2049 serum samples in 4 years (2003-2006) were collected and examined with IgG and IgM ELISA techniques. These subjects were in the age range of 20-35 years. Subjects were referred to No 1 Lab of Specialized Clinics of Tabriz University of Medical Sciences.
Results: Serum samples of 2049 females were analyzed, and 1814(%88.53) seropositive, 170 (8.29%) seronegative, 65 (3.17%) current infection were detected.
Conclusion: Findings showed that there was not a suitable programmed management or a confirmed diagnostic technique in our scientific society for the prevention of some of the side effects of CMV infection in fetus of pregnant women. For these reasons we must review and prepare a new strategy for guiding our female population at least in university scientific centers.

Keywords: *Cytomegalovirus, Congenital Disorders, ELISA technique.*
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INTRODUCTION

Cytomegalovirus (CMV) is a double–stranded herpes DNA virus that is transmitted by contact with infected blood, saliva, or urine, or by sexual contact. Vertical transmission of CMV may occur as a result of transplacental infection after primary or recurrent CMV infection, exposure to contaminated genital tract secretions at delivery, or through breastfeeding (1). Primary CMV infection occurs in 0.7% to 4.1% of pregnancies and the transmission rate varies between 24% and 75% (average 40%). CMV latent infections in the host may reactivate, and result in recurrent infection and fetal transmission occurrence in 0.5% to 1% of cases (2-4). Fetal transmission is not totally prevented by the presence of maternal antibodies to CMV, but this seems to have a protective effect against fetal disease, therefore the risk of the disease in children with maternal primary infection is higher than reactivated infection. In general, prenatal infections have more severe fetal consequences, when they occur early in gestation, because first trimester infections may disrupt organogenesis, while second and third trimester infections can cause neurological impairment or growth restriction. CMV can also be transmitted to the fetus when primary maternal infection occurs before conception, but data are not available about the consequences for the newborn under these

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circumstances. Congenital CMV infection can be the result of either exogenous or endogenous maternal infection. While exogenous infection can be primary or non-primary, as it can occur in both seronegative and seropositive women, endogenous infection is the result of reactivation of latent virus. As mentioned above, primary infection in mother has a much greater clinical impact on the fetus than recurrent infection or exogenous reification (5-11). For these reasons laboratory techniques represent a decisive diagnostic approach. There are many diagnostic problems due to CMV in pregnancy, and not all have been fully defined. The natural history of intrauterine CMV infection is not well understood, but it is clear that the viruses irreversibly cause damage to the fetus before delivery. These infants would not get much benefit from postnatal therapy, but if infection of the fetus could be detected before reaching this irreversible stage, treatment in utero (when available) might have a significant effect on the course of the disease (12). It has repeatedly been shown that isolation of viruses from amniotic fluid (AF) is effective in differentiating uninfected from infected fetuses (13,14). However, although Davis et al first reported prenatal diagnosis of congenital CMV infection in 1971, the number of reported cases is still low. In addition to the small number of cases of CMV infection diagnosed prenatally and the consequent limited experience with prenatal diagnosis, another important problem hampers this diagnostic aspect of CMV infection. It is difficult to determine which women should be enrolled in prenatal diagnostic programs. In fact, only pregnant women undergoing a primary CMV infection should be enrolled in prenatal diagnostic programs, and when seroconversion is not detected, the diagnosis of a primary infection is still problematic (15,16). Regarding to the complexities of clinical features, at the time of diagnosis, variability of diagnostic techniques, and outcome of infection, it seems that, there must be some essential educational programs for improving knowledge of the people, especially women at pregnancy ages. The following controversial issues are discussed in the light of the most recent advances in the following fields: the actual perception of the problem, universal serologic screening before pregnancy, the impact of correct counseling on decision making by the couple involved, the role of prenatal diagnosis in ascertaining transmission of virus to the fetus, the impact of preconception infection on the prevalence of congenital infection, and the prevalence of congenitally infected babies born to mothers who were immune prior to pregnancy compared to the number borne to mothers undergoing primary infection during pregnancy (17-20). In this research we tried to determine the immunity statues of females against CMV infection in the East Azerbaijani population, and then to evaluate reliability of available serologic techniques.

PATIENTS and METHODS

In this study, 2049 women in 4 years (2003–2006) referred to No1 Laboratory of Specialized Clinics of Tabriz University of Medical Sciences for determination of CMV (IgG and IgM) antibodies with ELISA technique. They were in the range of 20-35 years old. About 80% of subjects were from Tabriz and 20% from other cities of East Azerbaijan province (Ahar, Sarab, Azarshahr, Oskou). Serum samples were taken on their first attendance. But for the 75 pregnant cases blood samples were taken at the first attendance and again at intervals of one, two, three months until the end of gestation. The serum samples stored at–20◦ C, and were analyzed for CMV antibodies (IgG and IgM). All sera were sampled, stored, and tested under the same conditions. Briefly, the following procedures were performed:

All serum samples collected at the study were analyzed for IgG and IgM antibodies against CMV. For some of the IgG negative women, the last available serum of the pregnancy period was also

analyzed for IgG (RADIM–Italy) to detect seroconversion. For IgG test the optical density (OD) of each negative control and cut-off calibrator (10 RU/ml) was considered.

All serum samples collected at the study were also analyzed for IgM antibodies against CMV. For some of the IgM and IgG negative women the last available serum of the study period was also analyzed for IgM (RADIM–Italy) to detect seroconversion. For IgM test the OD of each negative, positive and cut- off was considered.

RESULTS

In this study a total of 2049 cases were surveyed for CMV (IgG and IgM) antibodies. There were 1814 (88.53%) seropositive (CMV IgG positive, CMV IgM Negative), and 170 (8.29%) seronegative (CMV IgG Negative, CMV IgM Negative) and 65 (3.17%) cases had current infection (CMV IgG positive, CMV IgM positive).

In 75 pregnant women whose pre-conception and post-conception serologic data were available, 5 cases had primary infection or seroconversion, 6 cases showed reactivation and 4 cases had current infection (table 1).

Table 1. Serologic patterns of pregnant women in accordance of pre and post conception data

Groups	No	No of IgG Pos	No of IgM Pos
Seoconversion	5(6.66%)	5	5
Recurrent infection	6 (8%)	6	-
Current infection	4(5.33%)	4	4
Seropositive	50(66.66%)	50	-

Table 2. Relationship between socioeconomic situation and CMV IgG specific antibody level

Socioeconomic Situation	Seropositive	Seronegative
High Income	68%	32%
Middle Income	75%	25%
Low Income	93%	7%

We observed a direct and indirect relationship between age and socioeconomic classes, and levels of CMV IgG specific antibody, respectively (tables 2 and 3).

Table 3. Relationship between age and CMV IgG specific antibody levels

Age Groups (Years)	IgG Levels (μg/mL)	No
20 - 25	80 - 85	986(52.4%7)
25 - 30	115 - 145	708(37.67%)
30 - 35	≥ 145	185(9.84%)

DISCUSSION

CMV is a DNA virus that Belongs to the group of herpes viruses. The prevalence of CMV infection varies according to socioeconomic background. In the United States the seropositivity rate is 50 - 60% for women of middle income, but it is 70 -80 % for those from lower socioeconomic sectors. In Europe, 45 % of pregnant women are seropositive at the beginning of pregnancy. Our study confirmed these facts, because we have found an indirect relationship between socioeconomic condition and seropositive state (table3). But there were a direct relation between age and levels of antibody in our study (table 4). Cytomegalovirus serum prevalence increases as age increases and reaches its maximum level after the age of 25. In fact, most of those women who had a previous history of cytomegalovirus infection were over 30 (21).

After the initial infection, CMV remains latent in host cells, and recurrent infection occurs following reactivation of latent virus. Prevalence of both primary and recurrent infection in pregnant women varies regionally from 0.7% to 4% for primary infection and up to 13.5% for recurrent infection. With advances in CMV serology, primary maternal infections which, until recently, were difficult to diagnose unless identified by seroconversion, can now be readily diagnosed by the presence of low avidity anti-CMV antibody, persisting for approximately 20 weeks after primary infection (22- 25). Collecting serum

samples for 3-4 weeks apart and testing them in parallel for anti-CMV IgG, is essential for the diagnosis of primary infection seroconversion from negative to positive. A significant increase (greater than 4 fold eg. 1:4to 1:16) in anti-CMV titers is the evidence of infection. The presence of CMV specific IgM is a useful but not completely reliable indication of primary infection. IgM titers may not be positive during an acute infection, or they may persist for months after the primary infection. Hence careful diagnosis of primary infection is required in the pregnant women based on the most sensitive serologic assays (IgM and IgG avidity). However, the final step for definite diagnosis of congenital CMV infection is detection of virus in blood or urine at the first 1 to 2 weeks of life (26, 27). Finally if we want to evaluate our findings and classify them, we must define some of the important explanatory variables, then compare the sensitivity or accuracy of diagnostic techniques and also determine what case, when and with which test should be analyzed.

Diagnosis of primary CMV infection is established when seroconversion is documented, i.e. the de novo appearance of virus-specific IgG in the serum of pregnant women who was previously sero negative. However, such an approach is feasible only when a screening program is adopted and seronegative women are identified and prospectively monitored. Regarding to these descriptions only 75 cases, whose pre and post conception serologic data were available and were followed up till the end of gestation and delivery, could be evaluated. Among them 5 cases were confirmed as primary infection, because before pregnancy they were seronegative, 2 cases of them exactly one month before pregnancy and 3 cases in the last trimester showed seropositivity. The 6 cases in which there were likely reactivations, previous IgG titers were in the range of 65 – 85 IU, but CMV specific IgG showed rising titers in the ranges of 120 – 145 IU during pregnancy. They were IgM negative, so it may be reactivation, or infection with other strains of virus. For these reasons for documentation of prenatal infection, amniotic fluid is the material of choice for determination of intrauterine virus transmission. The virus can be found by culture and or PCR of amniotic fluid. Both procedures can distinguish uninfected from infected fetuses, but cannot predict fetal outcome. Determination of viral load in amniotic fluid carried out by quantitative PCR is more promising approach and after further evaluation, may become an important starting point for future pre-emptive fetal therapy (28).

Thus IgM detection in the serum of pregnant women is likely to be a reliable marker; however IgM can reveal different clinical situations, which can be related to the acute phase of a primary CMV infection, the convalescent phase of a primary CMV infection, or the persistence of IgM antibody. The kinetic of the CMV-specific IgM antibody response during primary infection may vary greatly among individuals and depends substantially on the test or commercial kit used for testing (29). In general, high to medium levels of IgM antibody (peak titers) can be detected during the first 1 to 3 months after the onset of infection (acute or recent phase), after which the titer starts declining (convalescent or late phase) (30). Based on this kind of IgM interpretation there were not an observable IgG rising titers in 6 cases, but their IgM were positive for more than 6 months suspected for current infection. When the presence of CMV-specific IgM antibody in the serum of pregnant women cannot be directly related to a primary infection during pregnancy, an IgG avidity assay can help distinguish primary from non-primary CMV infection. This assay is based on the observation that virus specific IgG of low avidity is produced during the first months after onset of infection, whereas subsequently a maturation of process occurs by which IgG antibody of increasingly higher avidity is generated. Only IgG antibody of high avidity is detected in subjects with remote or recurrent CMV infection. Avidity level

are reported as the avidity index, expressing the percentage of IgG bound to the antigen following treatment with denaturing agents, such as 6M urea. The utility of the assay in diagnosing a primary infection has been reported for a variety of viruses. Measurement of IgG avidity is also valuable in determining the current or primary infection. In our study IgG avidity test seemed necessary for differentiation of current cases from recurrent or reactivation ones. The purpose of this study was to assess the accuracy of the serological tests especially the ELISA test. The results demonstrated that the serological tests had a low diagnostic performance in identifying CMV infection in pregnant women. Low diagnostic performance of serological tests means that a pregnant woman who is noncreative to IgM for CMV may still be undergoing viral replication through recurrent infection or viral reactivation (31). Approximately 5% of this population was susceptible to primary infection although they were IgG negative.

In conclusion, congenital CMV infection is a major health problem that should be approached on the basis of which woman should be enrolled in prenatal diagnostic program, which clinical specimen should be tested, and which laboratory procedure should be adopted for the diagnosis of congenital CMV transmission or infection. The importance may primarily be given to the introduction of antenatal screening programs for CMV infections in the developing countries.

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REFERENCES

1. HO M. Epidemiology of CMV infection. Rev infects Dis 1990;12(Suppl7): S701–10.

2. Stagno S, Pass RF, Cloud G, Britt WJ, Henderson RE, Walton PD, et al. primary cytomegalovirus infection in pregnancy, incidence, transmission to fetus, and clinical outcome. JAMA 1986;256:1904-8.

3. Davis LM, Tweet GV, Miller GN. Intrauterine diagnosis of CMV infection, viral recovery from amniocentesis fluid. Am J Obstet Gynecol 1971;109:1217–19.

4. Boppana SP, Rivers LB, Fowler KB, Mach M, Brith JB. Intrauterine transmission of CMV infection to infants of women with preconception immunity. N Engl J Med 2001;344:1366–71.

5. Henrich W, Mechis J, Vogel M, Enders G. Recurrent CMV infection during pregnancy. Ultrasound Obstet Gynecol 2002;19:608–11.

6. Alford CA, Stagno S, Pass RF, Britt WJ. Congenital and prenatal CMV infection. Rev infect Dis J 1999;12:5745–53.

7. Stagno S, Pass RF, Dworsky ME, Henderson RE, Moor EG, Walton PD, et al. Congenital CMV infection. The relative importance o f primary and recurrent maternal infection. N Eng J Med 1982;06:945–49.

8. Fowler KB, Stagno S, Pass RF. Congenital CMV infection. Risk in Future Pregnancies and Maternal CMV Immunity. 6th International CMV Workshop, Pediatric Beach Resort; Alabama USA; 1997.

9. Moreis DJ, Sims D, Shiswik M, Das WK, Newton VE. Symptomatic congenital CMV infection after maternal recurrent infection. Pediatr Infect Dis J 1994; 13:1361–64.

10. Indejeet G, Ramesh A, Shiv L, Shashi K. Congenital CMV in symptomatic infants in Delhi and surrounding area. Indian J Pediatr 2006;73:1095–97.

11. Conboy T, Stango S, Pass RF, Alford CA, Myers GJ, Britt WJ, et al. Early clinical manifestations and intellectual outcome in children with symptomatic congenital CMV infection J Pediatr 1987;111:343–48.

12. Whitley RJ, Cloud G, Gruber W, Storch GA, Demmler GJ, Jacobs RF, et al. Ganciclovir treatment of symptomatic congenital cytomegalovirus infection: results of a phase II study. National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. J Infect Dis 1997;175:1080-86.

13. Grose C, Wiener CT. Prenatal diagnosis of congenital CMV infection by virus isolation from amniotic fluid. Am J Obstet Gynecology. 1990;163:1253–55.

14. Hogge WA, Buffone GJ, Hogge JS. Prenatal diagnosis of cytomegalovirus (CMV) infection: a preliminary report. Prenat Diagn 1993;13:131–36.

15. Lamy ME, Mulongo KN, Gadisseux JF, Lyon G, Gaudy V, Van Lierde M. Prenatal diagnosis of fetal cytomegalovirus infection. Am J Obstet Gynecol 1992;166:91–4.

16. Lipitz SS, Yeggel E, Shalev R. Prenatal diagnosis of fetal CMV infection. Obstet Gynecol 1997;89:763–67.

17. Lynch IF, Daffos D, Mnuel G. Prenatal diagnosis of fetal CMV infection. Am J Obstet Gynecol 1991;165:714–18.

18. Corine L, Catherine D, Frachig G, Marie LD, Feredrich R. Prenatal diagnosis of congenital CMV infection. Obstet Gynecol 2000;95:881–888.

19. Clarissa SM, Ernani M, Alice BM, Maresa M. The prevalence of CMV infection in newborn, infants at an intensive care unites in a public hospital. J Pediatr 2006;82:46–50.

20. Stagno S, Pass RF, Dworsky ME, Henderson RE, Moore EG, Walton PD, et al. Congenital CMV infection: The relative importance of primary and recurrent maternal infection. N Engl J Med 1982;346:945–49.

21. Fowler KB, Stagno S, Pass RF. Maternal age and congenital cytomegalovirus infection: screening of two diverse newborn populations, 1980-1990. J Infect Dis 1993;168:552-56.

22. Fowler KB, Stagno S, Oass RF, Britt HJ, Alferd CA. The outcome of CMV infection in relation to maternal antibody statues. N Engl J Med 1992;326:663-67.

23. Blackburn NK, Besselar BD, Connel FO. Differentiation of primary CMV infection from reactivation using the urea denaturation test for measuring antibody avidity. J Med Virol 1991;33:6-9.

24. Ruellan-Eugene G, Barjot P, Campet M, Vabret A, Herlicoviez M, Muller G, et al. Evaluation of virological procedures to detect fetal human cytomegalovirus infection: avidity of IgG antibodies, virus detection in amniotic fluid and maternal serum. J Med Virol 1996;50:9-15.

25. Lazzarotto T, Spezzactera P, Pradelli P, Abate DA, Varrani S, Landini MP. Avidity of IgG directed against CMV during primary and secondary infections in incompetent and immunocompromised subjects. Clin Diagn Lab Immunol 1997;4:469–73.

26. Manna GR, Maurizeu Z, Milena F. Quantification of CMV DNA in amniotic fluid of mothers of congenitally infected fetuses. J Clin Microbiol 1999;37:3350–52.

27. Grose C, Wiener P. Prenatal diagnosis of congenital CMV infection by virus isolation after amniocentesis. J Pediatr Infect Dis 1992;11:605–7.

28. Revello MG, Baldanti F, Furione M, Sarasini A, Percivalle E, Zavattoni M, et al. Polymerase chain reaction for prenatal diagnosis of congenital human cytomegalovirus infection. J Med Virol 1995;47:462-66.

29. Dember G, Buffon C, Shimber N. Detection of CMV in urine of newborns of by using PCR. J Infect Dis 1988;158:1177-84.

30. Lazzarotto T, Dalla Casa B, Campisi B, Landini MP. Enzyme-linked immunoadsorbent assay for the detection of cytomegalovirus-IgM: comparison between eight commercial kits, immunofluorescence, and immunoblotting. J Clin Lab Anal 1992;6:216-18.

31. Kraat YJ, Stals FS, Christiaans MH, Lazzarotto T, Landini MP, Bruggeman CA. IgM antibody detection of ppUL80A and ppUL32 by immunoblotting: an early parameter for recurrent cytomegalovirus infection in renal transplant recipientsJ Med Virol. 1996 Mar;48(3):289-94.