CYTOMEGALOVIRUS INFECTION IN PRIMIPAROUS PREGNANT WOMEN AND THEIR NEONATES

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Abstract- ***Cytomegalovirus (CMV) is the most frequent cause of congenital infection in humans. In various parts of the world the prevalence of antibodies to CMV ranges from 40-100%. The prevalence of primary infection with CMV in pregnant Iranian women and risk of congenital CMV infection in their neonates are unknown. To determine the prevalence of CMV infection in primiparous pregnant (younger) women and incidence rate of congenital CMV infection among preterm and full-term infants borned from these women, in serum of 164 primigravid women before delivery, CMV IgG and IgM antibodies were measured by ELISA method and CMV-DNA detection by PCR in ~10% of their infants. 100% of women were immune to CMV infection (CMV-IgG positives). No acute infection (CMV-IgM positive) were detected in mothers and newborns. Therefore, we can not compare gestational age and weight of infants in seropositive and seronegative mothers. Probably, in Iranian pregnant women, CMV screening test is not recommended.***
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Key Words: Cytomegalovirus, congenital- CMV, CMV-antibody, TORCH screening

INTRODUCTION

Cytomegalovirus (CMV) is an ubiquitous organism that can cause infection at any time during the course of life. In various parts of the world, the prevalence of antibodies to CMV ranges from 40-100%. The rate of seropositivity in developed countries is 50% by the age of 50 years. CMV is the most common congenital infection, which occasionlly causes the syndrome of cytomegalic inclusion disease (hepatosplenomegaly, jaundice, petechia, purpura and microcephaly) (1,2). Beyond the neonatal period most primary infections are asymptomatic in the immunocompetent host, but like other members of the herpesvirus family, CMV can become latent (3-10). Primary infection occurs in a seronegative susceptible host. Recurrent infection represents reactivation of latent infection or reinfection in a seropositive immune host (11-30). Disease may results from primary or recurrent CMV infection, but the former infection is, more common cause of severe disease which increases with age, is higher in developing countries and among lower socioeconomic strata of the more developed nations (1,2). The incidence rate of congenital infection ranges from 0.2-2.4% of all live births, with the higher rates in populations with a lower standard of living (1-5). The fetus may become infected as a consequence of primary and recurrent maternal infection. The risk for fetal infection is greatest with maternal primary CMV infection (40%) and much less likely with recurrent infection (<1%) (1-5). The prevalence of primary infection with CMV in pregnant Iranian women and risk of congenital CMV infection in their neonates are unknown. Previous study determined CMV-IgG positivity in 98% and 100% of women less than 20 and over 40 years old in Tehran (32). The purpose of this prospective study was to assess the prevalence of primary infection with CMV in a population of primigravid (younger) women and the incidence rate of congenital CMV infection in their neonates.

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MATERIALS AND METHODS

This was a prospective multicenter study of primigravid women who were admitted in delivery ward of five educational and governmental hospitals with different geographic distribution in "Tehran, Iran" (Shohada in North, Bou-Ali in East, Hazrat-Rassol in West, Akbarabadi in South,and Firoozgar in central part of Tehran) between 1999-2000. One hundred and sixtyfive cases by multistage methods were selected randomly. Two ml blood was drawn from mothers on day of delivery and also from fetal surface of cord blood. Blood samples were centri-fuged and transferred to research laboratory. The

serum was restored in -20°C freezer until the serologic and PCR examination were performed on them.

Serological tests: The evaluation of anti-CMV IgG and IgM were carried out with commercial kits (Clone Systems EIAgen CMV IgG and IgM, Biochem Immuno Systems Italy. S. P. A.). Both kits were used and the results were interpreted as suggested by the manufacturer.

Results were calculated qualitatively. The ratio between the average O.D. value of the sample and that of the cut-off. The sample was considered, positive, if the ratio was > 1.1, doubtful, if the ratio was > 0.9 but < 1.1, negative, if the ratio was <0.9. If the results were doubtful, we repeated the test.

PCR: For 16 (9.8%) of the 164 samples, the viral genome was detected by PCR method. For DNA extraction, blood specimens were processed for isolation of leukocytes by density gradient centrifuge in Ficoll-Hypaque. Then, DNA was prepared by phenol-chloroform extraction protocol and was resuspended in 50 ml of sterile distilled water. Primers used in this study have been previously described (32). These primers bracket a 435 base pair sequence of human CMV (HCMV) DNA that codes for a major immediate early antigen of CMV.The primer sequences were:

P (1): 5'- CCAAGCGGCCTCTGATAACCAAGCC-3'

P (2): 5'- CAGCACCATCCTCCTCTTCCTCTGG-3'

Amplification condition: 5 µl of DNA sample was amplified in 100 µl of reaction mixture containing 50 mM Tris- Hcl (PH, 8.9), 50mM KCl, l6 mM $(NH_4)_2SO_4$, 7mM MgC12, 0.2 mg/ml bovine serum albumin, 0.25 mM deoxy ribonucleotide triphosphate (dNTPs) mixture, 0.5 µM of each primer, 0.03 units/µl of taq (Thermus aquesticus) polymerase (Pharmecia) and brought to a final volume of 100 in sterile distilled water. The reaction mixture was overlaid with 100 µl of mineral oil and boiled for 3 min and placed in a DNA thermal cycler (eppendorf, Mastercycler 5330). The amplification cycle consisted of a denaturation segment (94°C for 1 min) an annealing segment (55°C for 1 min) and an extention segment (72°C for 1 minutes). Amplification continued for a total of 32 cycles with a final extention at 72°C for 5 minutes. Amplified products were detected electrophoretically using a 1.5% agarose gel and visualized using ultraviolet fluorescence after staining for 15 min with ethidumbromide (10 mg/ml). To check the presence of inhibiting substances (false negative results) we used positive control containing DNA of HCMV.

RESULTS

The age of the studied mothers was 22.38± 4.47 (Range 14-42 years). Type of delivery: NVD/CS= 120/45. The occupation of the majority of mothers was housewife= 159 (96.4%), doctor= 1 (0.6%), student= 1 (0.6%), teacher= 2 (l.2%) missing= 1 (0. 6%); Place of birth of the mothers is shown in the table 1. Most of the father self financed their families. Three percent of neonates before or just after birth. In alive neonates, range of gestational age was 32-42 weeks, Mean= 36.56 ± 3.61 weeks, 80% of them aged between 39-40 weeks, four deliveries were twin; sex ratio was 85/80, mean weight of neonates was 3250 ± 1224.7 g. CMV-IgM was negative and CMV-IgG was positive in 100% of primigravid mothers. CMV-DNA detection by PCR method in cord blood of 16 samples (~10% of all) was performed. All of them were negative for CMV-DNA.

Table 1. The number (percent) of mothers in different living places in Tehran

Site of living in Tehran	Number	Percent
North	21	12.7
Central	20	12.1
East	43	26.1
South	58	35.2
West	23	13.9
Total	165	100

Table 2. The number (percent) of occupation of fathers

Husband job	Number	Percent
Self financed	87	25.7
Worker	38	23
Clerk	27	16.4
Driver	7	4.2
Teacher	2	0.12
Missing	4	0.24
Total	165	100

DISCUSSION

From a previous study in Iran (31), 98% of women less than 20 years and 100% in over 40 years in Tehran were CMV-IgG positive (mean= 99.1%). In the present study, all of primigravid were strongly " CMV-IgG positive" due to prior CMV infection, none of them were CMV-IgM positive (acute or recent infection) like other Iranian adults (31). Similar results were acquired in Brazil (85%) (34), Egypt (96%) (35) and Israel (84.3%) (36) and Iran (98%) (31). This study provides lower risk of primary infection with CMV in younger pregnant women compared with women in United States. No congenital CMV infection was revealed in the cord blood of 10% of these pregnancies (negative CMV-DNA detection by PCR method). According to these findings, probably screening of CMV infection in the period of pregnancy is not requested in Tehranian women.

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REFERENCES

1. Demler GJ. Cytomegalovirus. Congenital infections. In Feigin & Cherry textbook of Ped Inf Dis , 1998; vol (2); P: 1734-1737.

2. Sergio and Stagno. Cytomegalovirus. Remington and Klein. Inf Dis of the Fetus and Newborn Infants. 5th edi 2001; 3, 89.

3. Doyle M, Atkins JT, Rivera-Matos IR. Congenital cytomegalovirus infection in infants with Human Immunedeficiency Virus type 1. Ped Inf Dis J 1996; 15; 1102-1106.

4. Tufail A, Moe AA, Miler MJ, Wagar EA, Bruckner DA, Holland GN. Quantitative cytomegalovirus DNA level in the blood and its relationship to Cytomegalovirus retinitis in patients with Acquired Immune Deficiency Syndrome. Ophthalmology 1999; 106: 133-141.

5. Mitchell SM, Membrey WL, Youle MS, Obi A, Worrell S, Gazzard BG. Cytomegalovirus retinitis after the initiation of highly active antiretroviral therapy: a 2 year prospective study. Br J Ophthalmol 1999; 83: 652-655.

6. Fuchs AV, Wolf E, Scheider A, Jager H, Kampik A. Cytomegalovirus (CMV) retinitis in AIDS: ganciclovir implant in comparison to systemic therapy. Ophthalmologe 1999; 96: 11-5.

7. Boyrazlkiz HD, Witmer JP, Frissen PHJ. Cytomegalovirus (re)activation plays no role in the ocular vitritis observed after initiation of highly active antiretroviral therapy. AIDS 1999; 13: 867.

8. Whitley RJ, Holland GN. Cytomegalovirus retinitis -Evolving therapies in a new era. N Engl J Med 1999; 3(40): 1109-1110

9. Baumal CR, Levin AV, Read SE. Cytomegalovirus retinitis in immunosuppressed children. Am J Ophthalmol 1999; 127: 550-8.

10. Valantine HA. Prevention and treatment of cytomegalovirus disease in thoracic organ transplant patients: evidence for beneficial effects of hyperimmune globulin. Transplant Proc 1995; 27: 49.

11. Everrett JP, Hersberger RE, Norman DJ. Prolonged cytomegalovirus infection with viremia is associated with development of cardiac allograft vasculopathy. J Heart Lung Transplant 1992; 11.

12. Snydman DR, Werner BG, Heinze-Lacey B. Use of cytomegalovirus immune globulin to prevent cytomegalovirus disease in renal-transplant recipients. N Engl J Med 1987; 317: 1049-1054.

13. Cofer JB, Morris CA, Sutker WL. A randomized double-blind study of the effect of prophylactic immune globulin on the incidence and severity of CMV infection in the liver transplant recipient. Transplant Proc 1991; 23: 1525.

14. Welzl-Hinterkomer E, Tholen H, Sturmer J, Opravil M, Bernauer W. Bilateral cystoid macular edema following successful treatment of AIDS-associated CMV retinitis. Ophthalmology 1999; 96: 87-91.

15. Coats DK. CMV retinitis in two 10-month-old children with AIDS. J Pediatr Ophthalmol Strabismus 1999; 36: 109-110.

16. Lautensch L, Hckerstedt K, Jalanko H. Persistent cytomegalovirus in liver allografts with chronic rejection. Hepatology 1997; 25: 190.

17. Badley AD, Seaberg EC, Porayko MK. Prophylaxis of cytomegalovirus infection in liver transplantation. Transplantation 1997; 64: 66.

18. Gane E, Saliba F, Valdecasas GJ. Randomised trial of efficacy and safety of oral ganciclovir in the prevention of cytomegalovirus disease, in liver-transplant recipients. The Oral Ganciclovir International Transplantation Study Group [corrected] [see comments] [published erratum appears in Lancet 1998, 351: 454]. Lancet 1997; 350: 1729-1733. .

19. Winston DJ, Wirin D, Shaked A, Busuttil RW. Randomized comparison of ganciclovir and high-dose acyclovir for long-term cytomegalovirus prophylaxis in liver-transplant recipients. Lancet 1995; 346: 69.

20. Falagas ME, Paya C, Ruthazer R, Badley A, Patel R, Wiesner R, Griffith J, et al. Significance of cytomegalovirus for long-term survival after orthotopic liver transplantation -A prospective derivation and validation cohort analysis. Transplantation 1998; 66: 1020-1028.

21. Falagas ME, Snydman DR, Ruthazer R, Werner BG, Griffith J. Surveillance cultures of blood, urine, and throat specimens are not valuable for predicting cytomegalovirus disease in liver transplant recipients. Clin Inf Dis 1997; 24: 824.

22. Schmidt CA, Oettle H, Peng R. Comparison of polymerase chain reaction from plasma and buffy coat with antigen detection and occurrence of immunoglobulin M for the demonstration of cytomegalovirus infection after liver transplantation. Transplantation 1995; 59: 1133.

23. De Otero J, Gavalda J, Murio E, Vargas V, Calico L, Llopart L, Rossello J. Cytomegalovirus disease as a risk factor for graft loss and death after orthotopic liver transplantation. Clin Infect Dis 1998; 26: 865.

24. Falagas ME, Arbo M, Ruthazer R. Cytomegalovirus disease is associated with increased cost and hospital length of stay among orthotopic liver transplant recipients. Transplantation 1997; 63: 1595.

25. Merigan TC, Renlund DG, Keay S et al. A controlled trial of ganciclovir to prevent cytomegalovirus disease after heart transplantation. N Engl J Med 1992; 1182-1186.

26. Falagas ME, Snydman DR, Ruthazer R. Cytomegalovirus immune globulin (CMVIG) prophylaxis is associated with increased survival after orthotopic liver transplantation: The Boston Center for Liver Transplantation CMVIG Study Group. Clin Transplant, 1997; 11: 432.

27. Stratta RJ, Shaeffer MS, Markin RS, Cytomegalovirus infection and disease after liver transplantation. An overview. Dig Dis Sci 1992; 37: 673.

28. Noble S, Faulds D. Ganciclovir, an update of its use in the prevention of cytomegalovirus infection and disease in transplant recipients. Drugs 1998; 56: 115

29. Singh N, Yu VL, Mieles L, Wagener MM, Miner RC, Gayowski T. High-dose acyclovir compared with short-course preemptive ganciclovir therapy to prevent cytomegalovirus disease in liver transplant recipients. A randomized trial. Ann Intem Med 1994; 120: 375.

30. Fuchs AV, Wolf E, Scheider A, Jager H, Kampik A. Cytomegalovirus (CMV) retinitis in AIDS: ganciclovir implant in comparison to systemic therapy. Ophthalmology 1999; 96: 11-15.

31. Moddares S, Moddares Sh. Determination of CMV infection in infants and mothers in Tehran. 6th international congress of pediatrics, 15-20 Oct 1994, Tehran, Iran.

32. Demler GJ, Baffon M, Schimbor, RA May. Detection of cytomegalovirus in urine from newborns by using polymerase chain reaction DNA amplification. J Infect Dis 1998; 158: 1177-1184.

34. Sussuana Jh, Lete LL, Villela LH. Prevalence of CMV infection in different patient groups of an urban university in Brazil. Rev Soc Bra Med Trop 1995; 28(2): 105-108.

35. El Nawawy, Solliman A. Maternal and neonatal prevalence of toxoplasma and CMV antibodies in an Egyptian rural area. So J Trop Pedi tric 1996; 420): 154-157.

36. Stein O, Sheinberrg B, Schiff E, Mashiach S, Seidman DS. Prevalence of antibodies to CMV in parturient in Israel. Isr J Med Sci 1997; 33(l): 53-58.