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 primigravid 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. Acta Medica Iranica, 40(3); 136-139: 2002

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 occasionally 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

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 centrifuged and transferred to research laboratory. The
serum was restored at -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’- CCAAGCGGCTCTGATAACCAAGCC-3’
P (2): 5’- CAGCACCATCCTCCTCTTCCTGG-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, 16 mM (NH4)2SO4, 7mM MgCl2, 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 aquaticus) 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 extension segment (72°C for 1 minutes). Amplification continued for a total of 32 cycles with a final extension 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 ethidium bromide (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 (1.2%) 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.

**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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