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Congenital Cytomegalovirus Infection in Korean Population with Very High Prevalence of Maternal Immunity

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In order to asses congenital cytomegalovirus (CMV) infection in Korea, five hundred and seventy five pregnant women (mean age 29.5±3.8 yrs., mean gestational age at test 37.5± 6.7 weeks) visiting the prenatal clinic at Severance Hospital, Seoul, Korea were studied. CMV IgG antibody was present in 96% (552/575) and IgM antibody was present in 0.7% (4/575) of the pregnant women by the third trimester. Four of 445 cord sera were positive for CMV IgM antibody (0.9%). Urine samples from 514 newborns were tested for the evaluation of congenital CMV infection. Six (1.2%) of 514 newborns excreted CMV in their urine. All the congenitally infected infants had subclinical involvement at birth and during the 12 months of the follow-up period. These results indicate that Korean pregnant women were highly immunized against CMV by the third trimester. Furthermore this study suggests that the rate of congenital CMV infection is relatively as high as rates previously reported from other countries, although there is a very high prevalence of maternal immunity. The incidence of maternal primary infection during pregnancy seems to be rare and therefore most congenital infections in Korea might be following by maternal reactivation or reinfection.

Key Words: *Cytomegalovirus, Congenital infection*

INTRODUCTION

The incidence and outcome of congenital cytomegalovirus (CMV) are diverse, depending on many factors, especially socioeconomic factors and/or sexual behavior (Chandler et al., 1985). In South Korea, more than 80% of women have CMV antibodies by child bearing age but there are no published reports of the incidence of congenital CMV infection (Shim, 1981). Therefore, it can be deduced that the rate of primary infection in Korean pregnant women is low. However, a great number of infections can be said to be due to recurrent infections or reinfections. We have determined the incidence of congenital CMV infection by the screening of newborns for viuria and through serologic study for the CMV antibody by testing maternal sera and cord sera.

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Materials and Methods

Study Population: During the two years of this study, 575 women were screened for serologic evidence of CMV infection when they attended Severance and Yongdong Severance Hospital, Yonsei University College of Medicine for antenatal care, between Sep. 1989 and Aug. 1991. The serum was obtained during antenatal care together with the cord blood at the time of delivery. These sera were tested for the presence of CMV antibody. Five hundred and fourteen neonates were tested for congenital CMV infection by detection of virus from urine collected within three days of age.

Detection of CMV antibodies in serum: The sampled blood was immediately centrifuged and the obtained sera were frozen and stored at −20°C. CMV specific IgG and IgM antibody determination was performed by ELISA (Enzygost Behring, Germany). Rheumatoid factor was removed before performing IgM determi-

nation.

Identification of CMV in urine: The collected fresh urine specimens were inoculated into monolayer cultures of human fibroblast (HF) cells from newborn foreskins within 12 hours. To detect the presence of CMV, two kinds of murine monoclonal antibodies (Syva Company, Brussels, Belgium) against CMV specific major immediate-early antigen and early antigen were used. One milliliter of urine was centrifuged for 1 minute at 1500rpm and 0.15ml antibiotics (Gentamicin 50mg/ml + 10 unit of mycostatin/ml) were added to 0.45ml of the supernatant. The supernatant was left 15 minutes at room temperature. The sample was centrifuged again at 1500rpm for 1 minute and 100 microliter of the supernatant was inoculated with a monolayer of HF cells (9-10th passage). The fibroblast monolayer was precultured in 96 flat bottom microtiter plates, each well containing approximately 2×10^4 cells. After inoculation with urine, the microtiter plates were centrifuged at 1800rpm for 1 hour and fresh media was added. The plate was incubated for 16-24 hours, fixed with 100% ethanol, and incubated for 1 hour at 37°C with murine monoconal antibodies and goat anti-mouse IgG FITC (Kirkegaard & Perry Lab. Gaithersburg, USA). After culture, viral growth was identified by the presence of intranuclear fluorescence (Fig. 1). If positive results were obtained, urine culture was performed for virus isolation as in the conventionally described method (Reynolds et al., 1979).

RESULTS

The mean age of the 575 pregnant women included in the study was 29.5 years and the mean gestational age at testing 37.5 wks. The prevalence rate of CMV IgG antibody in the pregnant women was 96% (552/575). Four cases (0.7%) out of 575 pregnant women were positive for CMV IgM (Table 1). Four samples of 445 cord sera were positive for CMV IgM (0.9%). During the period of this study, 2389 newborns were delivered at Yonsei Medical Center.

Five hundred and fourteen neonates were screened for CMV in their urine and CMV was isolated from 6 neonates. The congenital infection rate was 1.2%. All

Table 1. Characteristics of the pregnant women in the test

No. of subjects	575
Age (Mean ± S.D.)	29.5 ± 3.8 yrs
Median age of mother	29 yrs
Gestational age at test	37.5 ± 6.7 wks
Positive rate of CMV IgG antibody	96% (552/575)
Positive rate of CMV IgM antibody	0.7% (4/575)

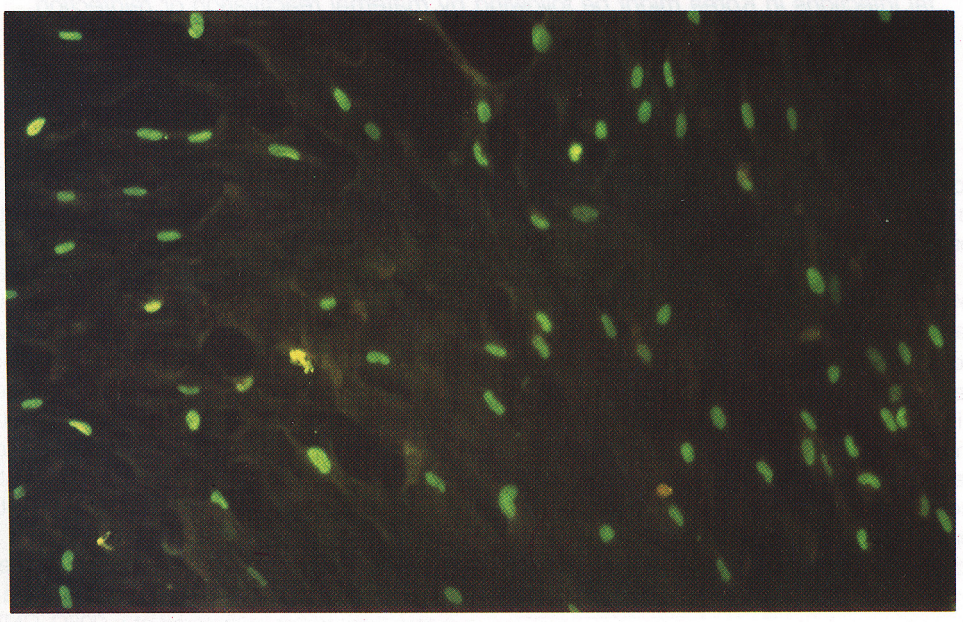

Fig. 1. Human fibroblast monolayers infected with newborn urine stained with FITC labeled monoclonal antibody of CMV immediate early antigen 24 hours following a centrifugation amplification inoculation procedure. (×100)

6 newborns with congenital infection were healthy at birth without clinical abnormality (Table 2). Clinical follow-up was performed on infants with congenital infection. At ages of 1, 2, 4, 6, 8 and 12 months, physical examination were performed. We were not able to detect any abnormal neurologic manifestations during the follow-up period.

Table 2. Characteristics of the newborns with congenital cytomegalovirus infection at birth*

Case No.	Maternal Age (yrs)	Gestational Age (wks)	Birth Weight (kg)	Clinical Manifestation
1	29	39	3.6	Normal
2	27	37	2.98	Normal
3	26	40	3.12	Normal
4	28	38	3.2	Normal
5	23	39	3.63	Normal
6	26	28	3.4	Normal

*6/514 neonates excreted CMV in their urine samples. 4/445 cord sera were positive for CMV IgM but virus was not isolated from these 4 neonates whose cord sera were positive for IgM.

DISCUSSION

The cytomegalovirus is the most common cause of congenital viral infection in humans, with a rate ranging from 0.5 to 2.2 percent of all live births in the United States (Stagno et al., 1981; Hanshaw et al., 1971). Congenital infections have been shown to follow recurrent or reactivated maternal infections as well as primary infections (Stagno et al., 1977). Although the mode of transmission of the virus from mother to fetus remains unclear, transplacental transmission is believed to result from viremia after primary maternal infection. Subsequent offspring would then presumably be protected from viral invasion by persistent maternal immunity. Maternal CMV infections, like all herpesvirus infections, regardless of pregnancy status, are commonly subjected to recurrences, especially in the genital tract. The role of these recurrent episodes in intrauterine transmission is poorly understood, notwithstanding the fact that they are more prevalent than primary involvement in women during the child bearing years. In women who do not have antibodies before pregnancy and in whom primary infection occurs during pregnancy, transplacental transmission of the virus will occur in 30-40% and in these cases more than 90% of the infants will be symptomatic with neurologic manifestations (Stagno et al., 1986; Stagno et al., 1982; Ahlfors et al., 1983). There has been no satisfactory explanation as to why some infants with congenital infections will have severe neurologic symptoms while others will be asymptomatic. According to the most recent reports, toxicity of the virus, type of infection of the mother during pregnancy (primary infection, recurrent infection, or reinfection) and time of infection seem to be the most important factors (Stagno et al., 1982: Ahlfors et al., 1983; Chandler et al., 1985).

Primary infection of the mother is closely related to the positive rate of antibodies within the group before pregnancy and the positive rate of CMV antibodies in women is related to the socioeconomic level. That is, in the higher socioeconomic group the appearance of CMV antibodies is less than in the lower socioeconomic group (50% vs 85%) giving rise to more congenital infections in the higher group (Stagno et al., 1977; Stagno et al., 1986). CMV infections in the lower socioeconomic group result in more recurrent infections and reinfections (Stagno et al., 1977). Women who had been exposed to CMV before pregnancy have antibodies (CMV IgG) within the serum. Congenital infection due to maternal recurrence is usually asymptomatic and the rate of neurologic manifestations is also very low (Conboy et al., 1986). This has been proven by restriction enzyme analysis with endonucleases where the CMV strains from the infected mothers and their infants were found to be the same (Huang et al., 1980). However, it has not been proven that reinfection by new strains results in congenital infection.

According to our study, 96% of pregnant women had CMV antibodies by the end of the third trimester. These results were compared with the prevalence rate of CMV antibody in other populations in Table 3. The prevalence rate of CMV IgG antibody screened by

Table 3. Prevalence rate of cytomegalovirus antibody in Seoul, Korea

Age of subject	No. positive/ No. tested	Percentage positive	Place
7-12 yrs	117/190	61.6*	School children[a]
12-13 yrs	30/46	65.2*	School girls[b]
16-40 yrs	242/300	80.6**	Blood doners[c]
16-40 yrs	104/120	86.6**	Child-bearing women[c]

*. CMV IgG antibody by ELISA; **, Complement fixing antibody

a, Cho YK, et al: Chung-Ang J Med 7:407-410, 1982
b, Kim CH, et al: Chung-Ang J Med 7:247-252, 1982
c, Shim YK: Korean J Epidermiology 3:99-104, 1981

Table 4. Rates of congenital cytomegalovirus infection in relation to the rate of maternal immunity in various locales

Location/Year	Congenital Infection (%)	Maternal Seropositivity (%)
Manchester, England/1978	0.24	25
Hamilton, Canada/1980	0.4	52
Birmingham, AL (USES*)/1981	0.6	60
Houston, Texas (LSES**)/1980	1.2	83
Abidjam, Ivory Coast/1978	1.4	100
Sendi, Japan/1970	1.4	83
Santiago, Chile/1978	1.7	98
Helsinki, Finland/1977	2.0	85
Birmingham, AL (LSES)/1980	2.2	85
Seoul, Korea/1991***	1.2	96

(Stagno S, Pass RF, et al. Maternal cytomegalovirus infection and perinatal transmission. Clin Obstet Gynecol. 1982, 25:563-576.) *USES: upper socioeconomic state, **LSES: lower socioeconomic state. ***Author's data.

complement fixing antibody test among blood donors in the Seoul area was 80.6% (Shim. 1981). On the other hand, the survey of primary school children in urban areas of Seoul revealed a 61.6% positive rate (Cho et al., 1982) and among girls from junior high school, 65.2% were positive for IgG antibody (Kim et al., 1982). The incidence of congenital infection is 1.2% (6/514) and all of the 6 infants with congenital infection were asymptomatic at birth. When compared with the data from other countries, the higher the rate of presence of maternal antibody, the higher was the rate of congenital infection (Table 4). In areas where the presence of maternal antibody was greater than 85%, the rate of congenital infection was 1.4 to 2.2% showing that the 1.2% rate of congenital infection obtained in this study is not remarkably high.

There are no data about the rate of seroconversion during pregnancy in the Korean population. And we can not understand the rate of primary congenital infections which will develop symptoms and sequelae. However, the prevalence rate of the IgM antibody during the third trimester was 0.7%.

In order to determine the presence of CMV infection, detection of the virus is the most accurate confirmative method. The presence of infection can be confirmed by detecting CMV antibodies, even though active infection cannot be determined. CMV IgG antibody nearly always means past CMV infection. Also, this state always precedes the presence of inapparent infection which may give rise to infection at any time. The presence of IgG antibody does not always mean the presence of "immune status". A rapid rise of the CMV IgG titer between two tests with an interval means present infection and a positive conversion indicates primary infection. However, the use of CMV IgM antibody by ELISA to determine the presence of neonatal CMV infection in practice has its limitations. First, the ELISA CMV IgM test is not as sensitive as viuria in detecting neonatal infection. The IgM antibodies were detected 1-7 days after the onset of illness, and titers reached a height of 1:64 or greater in 11 to 15 days, after which they gradually declined (Langenhusen et al., 1970). On occasion the response of CMV IgM antibody in individuals is different. Therefore, the absence of CMV IgM antibody does not imply the absence of CMV infection. In practive, 10-30% of the congenitally infected infants were negative for CMV IgM antibody but had viuria (Stagno et al., 1985; Griffits et al., 1982). Another importnat defect of the specific IgM test is that it is nonspecifically positive in EB virus infections and varicella-zoster infection (Ho. 1991). An IgM test may also be falsely positive in the presence of rheumatoid factor, which is an IgM antibody against IgG present in the blood of patients with certain collagen-vascular diseases and also frequently seen in pregnancy (Ho. 1991). In conclusion, it appears that the CMV IgM antibody test is ot very sensitive in detecting primary infection during pregnancy. False negatives are a problem that cannot be overcome by single serum tests. The most sensitive test is still seroconversion, which requires at least two samples. Four samples out of 445 cord sera were positive for IgM in this study but CMV was not isolated from these 4 neonates whose cord sera were positive for IgM. In the case of cord blood, care should be taken to avoid contamination by maternal blood, and it is prudent to confirm positive IgM antibody results by testing a follow-up specimen from the newborn.

Virus culture is the most reliable method for determining the presence of CMV infection. Current methods for identifying infected newborn infants involve tissue culture isolation of CMV, most commonly from urine or saliva. Conventional tissue culture isolation involves inoculation of the patient's sample onto a human fibroblast monolayer with frequent examination for characteristic cytopathic effect. More recently murine monoclonal antibodies reactive with CMV-encoded proteins have been used along with low speed centrifugation of the indicator monolayer to enhance the sensitivity and rapidity of CMV detection (Cleaves et al., 1982). This method helps to detect the presence of the virus within 48 hours of culture instead of the conventional method of waiting 4 to 6 weeks. If the

specificity of the monoclonal antibody against the early antigen is too high, the range of CMV strain to be detected will be too narrow.

Therefore, the monoclonal antibody used should contain the antigens of several CMV strains. The antibody used in this study was reacted with the immediate early protein of molecular weight 72,000 Dalton and early protein of molecular weight 50,000 Dalton produced from human CMV AD169. In this method, it is most important that maintenance of a fibroblast monolayer within the microplate. The human fibroblasts used in this study were obtained from neonatal foreskin and early passage was used (6-10th passages) and the density of the cell in each well was maintained at $2\text{-}5 \times 10^4$ cells.

The incidence of congenital CMV infection is 1.2% in Seoul, Korea, and most infants with congenital infection might have recurrent infections or reinfections. All the infants with congenital CMV infection had subclinical involvement without abnormal neurologic manifestation during the 1 year clinical follow-up. Although our follow-up were limited, maternal humoral immunity to CMV may provide substantial protection against damaging congenital CMV infection. Therefore, if preconceptual humoral immunity can prevent injury to the congenitally infected fetus, we can expect that CMV vaccine will protect against damage of the fetus from primary infection during pregnancy.

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