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IgG Avidity 검사를 이용한 한국인 산모에 있어 거대세포바이러스 일차감염의 혈청학적 선별

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Serologic Screening of Pregnant Korean Women for Primary Human Cytomegalovirus Infection Using IgG Avidity Test

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Background : Primary human cytomegalovirus (CMV) infection during pregnancy is a major cause of congenital malformation. We detected primary CMV infection in pregnant Korean women by using an algorithm that comprises CMV IgG, IgM, and IgG avidity tests.

Methods : During a 2-month period, 744 pregnant women who were at 10-19 weeks of gestation were consecutively enrolled in this study. Human anti-CMV IgG and IgM levels in their sera were determined by chemiluminescence immunoassays. Serum samples from the women who were positive for CMV IgG and IgM were assayed by the ARCHITECT CMV IgG avidity test in order to distinguish primary from non-primary CMV infection. Gross examination of the neonates of the women who were positive for CMV IgM was conducted.

Results : The seroprevalence of CMV IgG and IgM was estimated to be 98.1% and 1.7%, respectively. The samples from all the women who were positive for CMV IgM or with grey zone results contained high avidity CMV IgG. Seven women with positive CMV IgG and IgM results who completed follow-up up to delivery showed no gross evidence of in utero CMV transmission.

Conclusions : Maternal primary CMV infection was not detected in any of the pregnant women included in this study cohort. CMV IgG avidity test enabled the identification of women who were at a low risk of transmitting CMV infection and provided informative for subsequent pregnancy outcomes. Compared to previous studies, the seroprevalence of CMV IgG antibody across pregnant Korean women remained unchanged. *(Korean J Lab Med 2009;29:557-62)*

Key Words : *Cytomegalovirus, Congenital infection, Primary infection*

INTRODUCTION

Primary human cytomegalovirus (CMV) infection during pregnancy is one of the most common causes of congenital malformation [1]. In all, approximately 0.2-2.5% neonates worldwide are affected by congenital CMV infection [2]. Congenital CMV infection occurs via the intrauterine transmission of the virus. About 90% of the newborns infected during gestation are reported to be asymptomatic at birth, while others show varying degree of complications from hepatosplenomegaly, petechiae, and thrombocytopenia to more profound symptoms, including mental retardation, motor

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function disturbance, and hearing loss [3]. Even among the newborns without apparent clinical manifestations at birth, about 5–15% can develop delayed sequelae [3].

Because most infected pregnant women present only nonspecific symptoms, it is necessary to develop laboratory methods to diagnose primary CMV infection [4]. The usefulness of conducting serologic examination of maternal serum for CMV infection has been limited by the overlapping features between primary and non-primary infection, which does not cause severe congenital malformation and hence does not warrant specific intervention [5–7]. Therefore, it is important to distinguish primary infection from non-primary infection to provide targeted prenatal care to women who are at a high risk of transmitting CMV infection to their offspring. Recent advances in diagnostic methods have led to the development of reliable CMV IgG avidity tests that can detect primary CMV infection with a strong specificity of about 98% without compromising sensitivity [8]. This has also enabled the development of an algorithmic screening approach that incorporates CMV IgG, IgM, and IgG avidity tests to identify women who are at high risk of intrauterine transmission of the virus [4].

Using these serologic tests, it has become possible to easily obtain vital information on primary CMV infection. In Korea, although a few reports on CMV seroprevalence are available, there are no reliable data on the incidence of primary CMV infection during pregnancy [9, 10]. Hence, we aimed to estimate the frequency of primary CMV infection across pregnant Korean women using a recently developed CMV IgG avidity test.

MATERIALS AND METHODS

1. Materials

During a 2-month period, 744 pregnant Korean women at 10–19 weeks of gestation were consecutively enrolled for CMV serologic testing. Their serum samples were prospectively screened for CMV from November to December 2008. These women were visiting the primary women's clinic (Motaean Women's Hospital and Dana Women Clinic) for routine antenatal care. All the subjects provided a written informed consent. The mean age of the women enrolled in this study was 21.9±7.2 yr (mean±2SD).

Serum was separated from whole blood specimens obtained from the women, within 4 hr from collection. Serum specimens were maintained at 2–8℃ until serological assays were performed. Serological assays were not delayed for more than 10 days after blood collection.

2. Methods

CMV IgG and IgM were detected in the sera of all the women by using commercial chemiluminescence immunoassays (ARCHITECT *i*2000$_{SR}$; Abbott Laboratories, Abbott Park, IL, USA). ARCHITECT CMV IgG was a fully automated semiquantitative 2-step immunoassay incorporating viral lysate. Assay results equal to or more than 6.0 arbitrary units (AU)/mL were regarded as reactive. ARCHITECT CMV IgM was a qualitative 2-step immunoassay incorporating both viral lysate and recombinant antigen. An index value of 0.85–0.99 was regarded as a grey zone, while indices exceeding this value were interpreted as reactive. The grey zone results were treated in the same manner as the reactive results as previously described [8].

An automated IgG avidity assay (ARCHITECT CMV IgG Avidity; Abbott Laboratories, Abbott Park, IL, USA) was performed to distinguish between primary and non-primary CMV infections for cases that showed reactivity both with CMV IgG and IgM, provided adequate amount of sera was available. The CMV IgG avidity assay comprised two 2-step immunoassays performed using chemiluminescence technology. For each specimen, one aliquot of the sample was pretreated with CMV lysate that resulted in the selective binding and removal of the high-avidity antibody, while another aliquot was pretreated with the buffer without the CMV lysate. After the completion of the chemiluminescence reaction, the avidity of CMV IgG in the sample was calculated using the relative light units of both the tests. The abovementioned procedures were performed automatically once a sample was loaded onto the instrument. Results were obtained in percentages, and the gray zone was set to 50.0–

59.9% as per the manufacturer's instruction. Results higher than the gray zone indicated non-primary infection, while those lower than the upper limit of the grey zone indicated primary infection with a high risk of in utero CMV transmission. During the entire study period, the CMV serologic assays were performed in accordance with the routine quality control practice as per the relevant laboratory guidelines and manufacturer's recommendations.

For the women with detectable serum CMV IgM level, in utero CMV infection-related pregnancy outcome was evaluated on the basis of the existence of gross abnormality or growth retardation of their neonates at birth.

3. Statistical analysis

Student's *t* test was used for comparing between CMV IgM-seropositive and CMV IgM-seronegative women in terms of age, gestational age at the time of serologic testing, body weight, and concentration of CMV IgG. The differences were analyzed with MedCalc (MedCalc Software; Mariakerke, Belgium), and a *P* value of less than 0.05 was regarded as statistically significant.

Although ARCHITECT CMV IgM is a qualitative assay, a signal to cutoff ratio can be obtained for this assay. For evaluating the probability of false positivity of the assay, a negative δ value was calculated as logarithmic population means of signal to cutoff ratios divided by the logarithmic standard deviation of the signal to cutoff ratios [11]. The results for the women who showed grey zone reactivity on CMV IgM were not used for calculating the negative δ value, because these women were treated as seropositive in this study.

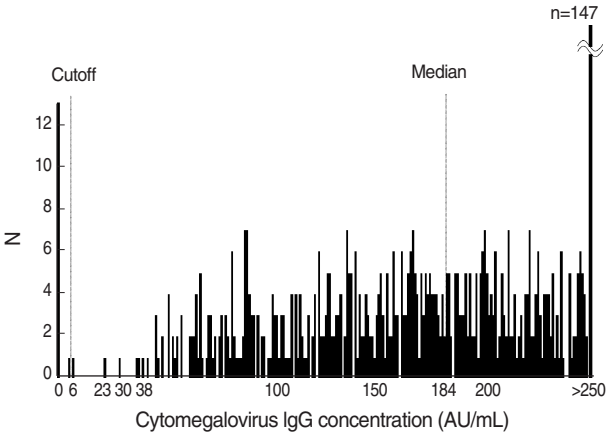

Fig. 1. Distribution of cytomegalovirus IgG concentrations in pregnant women.
Abbreviation: AU, arbitrary units.

RESULTS

Of the 744 pregnant women included in this study, 730 (98.1%) were seropositive for CMV IgG.

Median and 95% confidence interval of IgG concentration in the CMV IgG seronegative individuals were 0.3 and 0.10-0.58 AU/mL, respectively, while those in the seropositive ones were 183.8 and 165.7-191.9 AU/mL, respectively (Fig. 1). All the 14 samples that yielded negative results on the CMV IgG test were also negative on the CMV IgM test.

Distribution of the signal to cutoff values of the CMV IgM test were plotted (Fig. 2). In the CMV IgM test, samples from 10 women showed positive results and additional 3 samples showed grey zone results. All the CMV IgM-positive samples had a signal to cutoff ratio of less than 2.0. For the samples from individuals with negative results on the CMV IgM test, the median signal to cutoff value was 0.2 and the

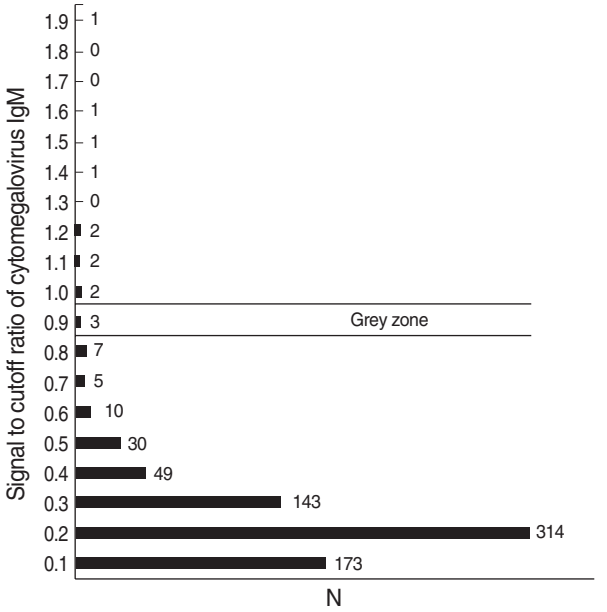

Fig. 2. Distribution of signal to cutoff ratios on the cytomegalovirus IgM test in pregnant women.

Table 1. Characteristics of the women with positive results both on cytomegalovirus IgG and IgM serological tests

Case no.	Age (yr)	Gestational age from LMP		Body weight (kg)	CMV IgG		CMV IgM		CMV IgG avidity		Neonatal exam*
		Weeks	Days		AU/mL	Interpretation	Signal to cutoff ratio	Interpretation	%	Interpretation	
1	31	16	4	47.5	87.9	Positive	1.6	Positive	85.0	High	Normal
2	37	17	2	58.2	>250.0	Positive	1.5	Positive	94.7	High	Normal
3	27	17	2	54.0	92.4	Positive	1.1	Positive	88.5	High	Normal
4	36	15	0	53.7	101.0	Positive	1.0	Grey zone	65.2	High	Normal
5	39	17	0	52.4	247.4	Positive	0.9	Grey zone	81.2	High	Normal
6	26	17	4	61.2	>250.0	Positive	1.9	Positive	97.7	High	Not done
7	28	16	1	63.5	237.9	Positive	1.4	Positive	98.1	High	Not done
8	34	16	1	48.0	184.6	Positive	0.9	Grey zone	91.3	High	Normal
9	24	16	2	99.0	94.2	Positive	1.2	Positive	98.2	High	Not done
10	33	16	3	44.1	>250.0	Positive	1.0	Positive	74.6	High	Not done
11	26	16	2	59.3	167.0	Positive	1.0	Positive	90.0	High	Not done
12	23	15	0	64.0	140.1	Positive	1.1	Positive	80.6	High	Normal
13	28	15	3	79.0	>250.0	Positive	1.2	Positive	Not done		Not done

*Neonatal exam, gross examination at birth.
Abbreviations: LMP, last menstrual period; CMV, cytomegalovirus; AU, arbitrary units.

negative δ value was estimated to be −3.1. All the women with positive or grey zone results on CMV IgM also showed positive results on CMV IgG (87.9~>250 AU/mL), and their demographical and serological characteristics are summarized in Table 1. Compared with the women who showed positive results exclusively on CMV IgG, those who showed positive results both on CMV IgG and CMV IgM showed no statistically significant difference in terms of age, gestational age at the time of serologic testing, body weight, and concentration of CMV IgG. The CMV IgG avidity test was performed on samples from 12 women who showed positive results both on CMV IgG and CMV IgM tests. All the 12 samples showed high CMV IgG avidity index (65.2–98.2%), and these women were determined to be at a low risk of transmitting CMV infection (Table 1). The IgG avidity test was not performed for 1 patient owing to the lack of sufficient sample amount. Of the 12 women for whom the CMV IgG avidity results were available, the pregnancy outcomes of 7 could be evaluated (Case 1–5, 8, and 12). None of the neonates of the 7 women showed evidence of congenital CMV infection on routine neonatal examination. Owing to the lack of apparent indications, specific tests to rule out congenital CMV infection were not performed.

DISCUSSION

Primary CMV infection during pregnancy is known to be associated with the development of congenital malformation with a 15–25% probability [3]. Although the risk involved in recurrent maternal CMV infections cannot be neglected, the possibility of serious sequelae such as neurologic manifestations is thought to be very low [12]. Therefore, the risk of congenital CMV infection was thought to be closely related to the history of the infection in mothers, thereby resulting in the existence of antibodies against CMV before pregnancy [1]. In this study, 98.1% of the pregnant women had CMV IgG. Seroprevalence of CMV IgM was estimated to be 1.7%, provided the grey zone was included. Compared to the results of previous studies, seroprevalence of CMV antibody across pregnant Korean women has not changed significantly: it was 98.9% in 1984 and 96.0% in 1992 [9, 10]. Seroprevalence of CMV IgG obtained in this study was similar to that in Japan (94%), but was higher than that in the United States (50–88%), the UK (56–60%), and Italy (70%) [13].

On the basis of the recommended cut off value, a negative δ value of −3.1 on the CMV IgM assay was thought to be acceptable to distinguish seronegative individuals from the seropositive ones. Positive δ values could not be cal-

culated owing to the limited number of positive specimens.

Thus far, there have been no data on the frequency of primary CMV infection during pregnancy in Korea due to the lack of an accessible method to identify primary CMV infection. PCR of CMV isolated from the amniotic fluid could be a feasible approach. However, its usefulness was shown to be significantly limited by a low diagnostic sensitivity of 30-45% prior to 21 weeks of gestation and its innate invasiveness [4].

Presently, the CMV IgG avidity assay seems to be one of the most accessible tools to differentiate primary from non-primary CMV infection with substantial reliability in patients with CMV IgG- and IgM-positive sera. A clinical study on 31 pregnant women with recent primary CMV infection revealed that both the currently available automated CMV IgG avidity assays, including the ARCHITECT CMV IgG Avidity assay, could identify all the pregnant women whose CMV IgG was seroconverted within the past 4 months [8]. More specifically, in their study, ARCHITECT CMV IgG Avidity showed a low level in all the 25 cases whose CMV IgG was converted from negative to positive within the past 4 months but changed to a high level after 40 weeks, implying its applicability to detect primary CMV infection in pregnant women [8].

Because of the high sensitivity for identifying primary CMV infection, CMV IgG avidity tests have been occasionally used in the past. However, wide ranges of the grey zone (up to 60%) and incorporation of manual steps in the previously available assays hindered their wide acceptance in clinical laboratories [8]. In addition, the interpretation criteria recommended by the manufacturers were not always acceptable for evaluating primary infection [4]. However, the ARCHITECT CMV IgG Avidity has significantly addressed these issues. According to a previously published report, of the 100 patients with non-primary CMV infection, 24% failed to show high level CMV IgG avidity with a previously available alternative immunoassay, while 98% showed high CMV IgG avidity using ARCHITECT [8]. Because ARCHITECT CMV IgG Avidity has a fully automated assay procedure and a narrow grey zone of only 10%, the authors could reliably identify women with primary infection in a time-dependent manner using the unmodified interpretation criteria provided by the manufacturer [8]. In this study, low avidity or grey zone avidity was not observed among the 12 CMV IgG- and IgM-positive pregnant women who were tested by the CMV IgG avidity test. All the women whose samples were tested with CMV IgG avidity were thought to be at a low risk of transmitting CMV infection to their offspring, as reflected by the grossly normal neonates of the 7 women who completed follow-up up to delivery.

Incorporation of the CMV IgG avidity test was beneficial to determine the CMV infectious status without the requirement of further invasive diagnostic procedures. This might possibly reduce the healthcare expenses incurred. Moreover, women with high CMV IgG avidity indices could maintain their pregnancy without the concerns of transmitting CMV infection to their offspring.

In this study, the neonates of the women who tested positive for the virus were examined non-specifically for evaluating congenital CMV infection because of the minimal level of perceived risk. No visibly identifiable sequela of in utero CMV infection was recognized among the 7 cases that were followed-up up to delivery.

In Korea, although national surveillance system or authorized recommendation for population-based screening has not yet been performed, congenital CMV infections with serious sequelae have been posing as a serious issue [14, 15]. Although rubella was considered as the most significant pathogen that causes congenital malformation, increasing evidence of universal vaccination will eliminate most of rubella-induced congenital malformation cases. In contrast, congenital CMV infection remains a serious issue owing to the relatively low level of awareness and absence of vaccination. In Korea, serologic screening is not widely used for maternal CMV infection and no consensual standards of prenatal care have yet been established. Our study indicates that CMV screening in pregnant women would probably gain more importance in the future antenatal care, and the diagnostic value of reliable and readily accessible serologic tests would be recognized in the future. This study also showed that the automated serological screening incorporating CMV IgG avidity test enables the easy identifica-

tion of individuals with non-primary CMV infection and can efficiently differentiate pregnant women at a lower risk of intrauterine CMV transmission from those at a higher risk.

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