

Primary Cytomegalovirus Infection in Pregnant Egyptian Women Confirmed by Cytomegalovirus IgG Avidity Testing

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Key Words

Primary cytomegalovirus · Pregnancy · Avidity

Abstract

Objective: To determine the frequency of primary cytomegalovirus (CMV) infection in pregnant Egyptian women using CMV IgG avidity testing. **Subjects and Methods:** A cross-sectional study was conducted at Suez Canal University Hospital, Ismailia, Egypt. A total of 546 pregnant women, presenting for routine antenatal screening, were tested for CMV IgG and IgM using a commercially available enzyme-linked immunosorbent assay (ELISA). Sera from CMV IgM-positive women were tested by CMV IgG avidity assay. **Results:** All the 546 pregnant women were seropositive for anti-CMV IgG. Of the 546 women, 40 (7.3%) were positive or equivocal for IgM antibodies. All sera from the 40 women (IgG+/IgM+) showed a high or intermediate CMV IgG avidity index. Of the 40 women, 23 (57.5%) were in the second or third trimesters of pregnancy and had their first-trimester blood retrieved, and the tested CMV IgG avidity assay showed a high avidity index. **Conclusion:** Women who were IgM positive had no primary CMV infection in the index pregnancy as evidenced by the high CMV IgG avidity testing. © 2013 S. Karger AG, Basel

Introduction

Human cytomegalovirus (CMV) is the most common cause of congenital malformations resulting from viral intrauterine infection in developed countries [1]. Primary CMV infection occurs in 0.15–2.0% of all pregnancies and may be transmitted to the fetus in up to 40% of cases [2]. In developing countries, reliable estimates of prevalence and outcome of primary CMV infection are not available, and studies from Middle Eastern countries have reported a high seroprevalence of CMV IgG in pregnant women and a low prevalence of CMV IgM [3–7]. However, in the majority of these studies, no further diagnostic evaluation was carried out in women who were CMV IgM positive to determine whether this was due to primary or past CMV infection.

Primary infection is defined as CMV infection in a previously seronegative person; subsequently, the virus becomes dormant and exists in a latent state, from which it can be reactivated causing recurrent (secondary) infection [8]. In addition to viral reactivation, there seem to be several strains of CMV that infect humans, so that reinfection can occur even in immunocompetent individuals [9]. The accuracy of maternal anti-CMV IgM to predict

primary maternal infection is complicated by the fact that IgM antibodies can persist for months or even years after primary infection and can also be found in the setting of reactivation or reinfection with a different strain of CMV [9].

Moreover, false positive CMV IgM results can occur in the course of non-CMV illnesses such as primary Epstein-Barr virus infection due to potent B-cell stimulation. Furthermore, studies have demonstrated a high variability in specificity and sensitivity among CMV IgM assays in addition to a high rate of discordance [10, 11]. Thus, testing for IgM, particularly in asymptomatic pregnant women, may frequently create a problem rather than solving it.

As primary CMV infections during pregnancy are associated with a high risk of virus transmission to the fetus, discrimination between recent primary and past CMV infection can be an important tool in the clinical management of pregnant women [9].

Recently, it has been shown that antibody avidity, which is an indirect measure of the tightness of antibody binding to its target antigen, increases in the first weeks after a primary infection. Low-avidity IgG antibodies to CMV persist for up to 20 weeks after a primary CMV infection [12]. These low-avidity antibodies are then replaced by high-avidity antibodies. Currently, the combination of the presence of anti-CMV IgM antibodies and low-avidity anti-CMV IgG antibodies along with maternal or fetal symptoms is used for the diagnosis of a primary maternal infection [13].

The prevalence of primary maternal CMV infection is not known in Egypt due to factors such as the lack of national screening for CMV to identify those who are seronegative and the absence of an accessible method to identify primary CMV infection. Therefore, in the present study, we aimed to determine the risk of primary CMV infection by using the CMV IgG avidity test in a sample of women from Suez Canal Area, Egypt.

Subjects and Methods

Study Population and Specimens

A cross-sectional descriptive study was conducted from June 2011 to October 2012. Questionnaires were used to gather sociodemographic data. A total of 546 pregnant women who presented for routine antenatal care at the Obstetrics and Gynecology Department, Suez Canal University Hospital, Ismailia, Egypt, were included in this study. Whole blood samples were collected, centrifuged at 2,000 g for 15 min, and sera were removed and stored at -20°C . The samples were coded by date of collection and sample number.

Table 1. Results of CMV IgM and IgG (by ELISA) in pregnant women by trimester

Trimester	Subjects, n	IgM+/IgG+	IgM-/IgG+
1st	270	17 (6.3%)	253 (93.7%)
2nd	66	6 (9.1%) ^a	60 (90.9%)
3rd	210	17 (8.1%) ^a	193 (91.9%)
Total	546	40 (7.3%)	506 (92.7%)

^a Twenty-three women in the second and third trimester who were positive for CMV IgM and IgG had their first-trimester blood retrieved and tested by CMV IgG avidity.

Ethical approval to perform the study was obtained from the Ethics Committee, Faculty of Medicine, Suez Canal University. Informed consent was obtained from all women prior to the collection of specimens.

Serology

The CMV IgM and IgG were screened in patients' sera by using a commercially available capture enzyme-linked immunosorbent assay (ELISA) (CMV IgM and CMV IgG, Dia.Pro; Diagnostic Bioprobes Srl, Italy).

Samples with concentrations >0.5 IU/ml (WHO) were considered positive for anti-CMV IgG antibody, whereas samples were considered IgM positive when the ratio of the sample optical density at 450 nm to the cutoff value (signal to cutoff) was >1.2 and were considered equivocal when the ratio was between 1 and 1.2.

Any CMV IgM-positive woman was then tested by an IgG avidity assay (VIDAS CMV IgG avidity; BioMérieux, Lyon, France). VIDAS CMV IgG avidity assay (enzyme-linked fluorescent assay, ELFA) consists of two assays with and without 6 M urea to dissociate low-avidity antibodies, which enables weak-avidity antibodies produced at the early stage of a primary infection to be differentiated from high-avidity antibodies, which are characteristic of a former infection. The procedures and interpretation of results were performed according to the manufacturer's instructions.

The avidity index is the ratio of the relative fluorescence value obtained for the sample with the strip containing urea buffer to the relative fluorescence value without urea buffer. On the VIDAS instrument, an avidity index ≥ 0.8 is a strong indicator of a primary infection dating back >3 months, while an index <0.2 is a strong indicator of a primary infection dating back <3 months. An avidity index between 0.2 and 0.8 is not capable of distinguishing a recent from a past infection. ELISA assay results were analyzed in the three trimesters of pregnancy for all women included in the study.

Results

Of the 546 pregnant women involved in this study, all (100%) were seropositive for anti-CMV IgG. Of those women, 40 (7.3%) were positive for IgM antibodies (17 di-

Table 2. Characteristics of women with positive results for both CMV IgM and IgG serological tests

Case No.	Age, years	Gestation at testing, weeks	CMV IgM	CMV IgM S/Co	CMV IgG avidity	Neonatal outcome
1	20	10	positive	1.2628	high	–
2	26	19	positive	1.2696	high	–
3	22	18	gray zone	1.041	high	–
4	26	11	gray zone	1.071	high	–
5	20	37	positive	1.2538	high	normal
6	36	11	positive	1.2491	intermediate	–
7	27	36	positive	2.4182	intermediate	normal
8	29	12	positive	1.5041	high	–
9	21	21	gray zone	1.1051	high	–
10	20	33	positive	2.1064	intermediate	–
11	30	11	positive	3.8874	high	–
12	30	10	positive	1.5973	high	–
13	28	38	positive	2.9181	high	normal
14	21	38	positive	1.7645	intermediate	normal
15	24	36	gray zone	1.1945	high	normal
16	28	11	positive	2.2218	high	–
17	19	10	positive	3.2526	high	–
18	30	24	positive	1.3127	high	–
19	30	25	positive	1.2984	high	–
20	25	34	positive	1.2914	high	normal
21	27	10	gray zone	1.0885	high	–
22	25	12	positive	2.1352	high	–
23	23	35	positive	2.3589	intermediate	normal
24	25	36	gray zone	1.1205	high	normal
25	21	12	positive	1.9438	high	–
26	35	37	positive	2.2674	high	normal
27	25	10	positive	3.1729	intermediate	–
28	30	30	positive	2.3859	high	–
29	22	39	positive	1.7295	high	normal
30	21	22	positive	1.8329	intermediate	–
31	31	30	positive	1.7649	high	–
32	31	36	positive	1.2437	high	normal
33	27	9	positive	2.1262	high	–
34	20	11	positive	2.2158	intermediate	–
35	25	33	gray zone	1.1319	high	–
36	24	25	positive	2.3156	high	–
37	20	10	positive	1.4312	high	–
38	31	12	positive	1.2385	high	–
39	29	32	gray zone	1.0779	high	–
40	24	11	positive	3.2196	high	–

S/Co: Ratio of the sample optical density at 450 nm to the cut-off; high avidity index: >0.8; intermediate avidity index: 0.2–0.8; low avidity index: <0.2; –: no available data.

agnosed in the first trimester, 6 in the second trimester, and 17 in the third trimester). The results of IgM and IgG ELISA assays of all women included in the study divided into three groups (group A: first trimester; group B: second trimester, and group C: third trimester) are given in table 1.

Table 3. CMV IgG avidity results in pregnant women positive or gray zone by ELISA IgM anti-CMV antibodies assay

CMV IgM positive/gray zone	IgG avidity		
	high	intermediate	low
1st trimester	14	3	0
2nd trimester	5	1	0
3rd trimester	13	4	0
Total	32	8	0

High avidity index: >0.8; intermediate avidity index: 0.2–0.8; low avidity index: <0.2.

The mean age of the study population was 26.6 ± 2.6 years (range 16–43), with no significant difference seen between the mean age of women with negative or positive CMV IgM ($p = 0.9$). Women positive for CMV IgG showed a non-statistically significant difference in terms of gestational age at the time of serologic testing compared to those who had positive results both for CMV IgG and CMV IgM ($p = 0.65$). The median number of pregnancies was 3 (range 1–9).

In the CMV IgM test, samples from 32% of the women showed positive results and an additional 8% of samples showed grey-zone (equivocal) results. The demographic and serological characteristics for all women with positive or grey-zone results on CMV IgM are summarized in table 2.

The CMV IgG avidity test performed on samples from all 40 women who had positive results on both CMV IgG and CMV IgM tests provided the grey zone as illustrated in table 3. All the 40 samples showed a high and intermediate CMV IgG avidity index. Of these women, 23% were in the second or third trimesters and had their first-trimester blood retrieved and tested which showed the same results.

Discussion

In this study, all pregnant women had CMV IgG. The estimated seroprevalence of CMV IgM was high, indicating the likelihood of an endemic nature of infection in the Suez Canal Area of Egypt, and confirmed the ubiquitous past exposure to infection in the Suez Canal area. Our results also confirmed data of previous studies from Egypt and other Middle Eastern countries [3, 6, 14, 15]. In a study from Iran by Bagheri et al. [3], the majority of pregnant women (72.1%) were positive for CMV IgG and

2.5% were positive for CMV IgM. In Gaza strip, Palestine [4], the anti-CMV IgM was 6% among pregnant females, whereas in Turkey, the positivity for anti-CMV IgG antibody was 97.3%, while 1% were positive for anti-CMV IgM [5]. CMV total IgG antibodies were found in 92.1% in Saudi Arabia [6]. In a study in Egypt [14], there were no cases of primary CMV diagnosed in 132 pregnant women, and only 4.5% of these women were diagnosed with recurrent CMV infection, which was defined as being IgG positive prior to pregnancy, IgG positive at the first pregnancy visit without IgM test, or IgM positive with high IgG avidity. In Sudan [15], it was reported that CMV seroprevalence among antenatal women was 84%.

The present study was carried out at a University Hospital which is characterized by a high rate of attendance of patients with low socioeconomic status. A previous study [16] found a correlation between the socioeconomic status within a community and risk of CMV infection.

In the present study, the mean age of the studied women was 26.6 years. In another large cross-sectional study, maternal age of 25 years and more was found to be associated with less congenital CMV infection according to Fowler et al. [17].

Thus far, there have been no data on the frequency of primary CMV infection among the minority who are IgM positive during pregnancy in Egypt due to the lack of an easy-to-use method to identify primary CMV infection. Polymerase chain reaction of CMV isolated from the amniotic fluid could be a feasible approach [18]. However, its usefulness was shown to be limited due to its invasive nature and a low diagnostic sensitivity of 30–45% prior to 21 weeks of gestation [19].

Currently, the CMV IgG avidity assay seems to be one of the most accessible tools to differentiate primary from non-primary CMV infection [20]. In our study, the use of the CMV IgG avidity test was useful in excluding primary CMV infectious status without the need of further invasive diagnostic procedures. This might possibly lessen the healthcare expenses incurred. Moreover, women with high CMV IgG avidity indices could maintain their pregnancy with a lower risk of transmitting CMV infection to their offspring [13].

In our screening plan, we included women who were more advanced in gestation and, by retrieving and testing the first-trimester blood, showed high CMV IgG avidity, thus indicating the possibility of applying this assay to pregnant women in the second and third trimester to screen for low antibody avidity which is associated with a higher risk of fetal transmission [21].

The limitations of this study included the fact that we did not use polymerase chain reaction to detect viral DNA in IgM-positive cases in order to detect the prevalence of active infection, and the lack of follow-up for pregnancy outcomes to exclude CMV transmission to the fetus.

Conclusion

IgM-positive women in this study showed a low risk of primary CMV infection as indicated by high IgG avidity testing. This was the first study in Egypt utilizing IgG avidity testing to differentiate between primary and non-primary infection in CMV IgM-positive patients.

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