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Microbiologia Medica; volume 31:4914

Value of immunoglobulin G avidity for primary cytomegalovirus diagnosis in pregnant women compared to nested polymerase chain reaction

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Summary

Primary human cytomegalovirus (HCMV) infection in pregnancy especially in the first term carries high risks to foetus. Diagnosis depends mainly on laboratory tools as clinical diagnosis is non specific. The aim of the present study was to detect recent infection with HCMV in cohort pregnant Egyptian women in the first trimester by serological detection of specific IgM, IgG and IgG avidity test compared to nested PCR method. This study was performed on consecutive pregnant women. Blood samples were obtained and sera were separated from 120 pregnant women in their first trimester of pregnancy and were screened for anti-CMV IgG, IgM and IgG avidity by ELISA and for HCMV-DNA by PCR. Positive IgG for HCMV was 62.5% and positive IgM was 15%. IgG avidity results showed that low, intermediate and high avidity represented 33.3%, 16.7% and 50%, respectively of IgM positive patients and 42.1%, 38.6% and 19.3%, respectively of IgM negative patients. Detection of HCMV DNA by PCR was positive in one of 24 IgM negative/low IgG avidity, in three of 22 IgM negative/intermediate IgG avidity and in all IgM positive/low IgG avidity patients. Confirmatory testing for primary infection with the combined use of IgG avidity with IgM antibody test in pregnant women during the first trimester is an accurate screening tool. The use of PCR as a diagnostic method can be applied to pregnant women with IgM antibodies and low IgG avidity results or in presence of isolated low or intermediate avidity IgG.

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Key words: Cytomeglovirus; Egyptian women; polymerase chain reaction; IgG avidity.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 16 December 2014.
Revision received: 2 February 2016.
Accepted for publication: 6 February 2016.

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Microbiologia Medica 2016; 31:4914
doi:10.4081/mm.2016.4914

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Introduction

Cytomegalovirus (HCMV) belongs to the herpes virus family. It is known as β-herpes. It is distributed universally in various geographic locations (10). HCMV infection is usually asymptomatic in immunocompetent subjects with no long term sequel. On the other hand, HCMV infection is important in pregnant women due to their immunocompromised state and risk of infection to the foetus.

As a member of herpes virus family the infection of HCMV is latent infection the virus remains dormant within the individual's body for life. Recurrent disease occurs in immunocompromised conditions. It was reported that the risk of foetal damage is greater if the primary infection occurs during the first trimester of pregnancy. Prevalence of congenital infection ranges from 0.2% to 2.5% in different populations (1,6,7,12,17,21,22). The seroprevalence of HCMV among women of childbearing age varies from 35% to 95% in different countries (4,11,20,27). It is more widespread in developing countries and in areas of lower socioeconomic conditions (16).

Primary HCMV infection acquired during pregnancy has the main risk for congenital infection with intrauterine transmission of the cytomegalovirus occurring in around 40% of infections with 10% of the live-born infant is affected by symptomatic disease at birth and later. In addition, 10-15% of the asymptomatic new-born develops late sequels such as sensor neural hearing loss and neurodevelopmental disorders (23,26).

The diagnosis of primary cytomegalovirus infection in pregnant women is difficult as the infection is clinically silent or accompanied by non-specific symptoms like headache, fever, or flu-like symptoms. Serological assessment of specific antibodies toward HCMV is not sufficient. Supplementary serologic assays such as the specific IgG avidity enzyme immunoassay (EIA) or the micro neutralisation test have been used to differentiate between primary and recurrent or past infections and have been shown to be useful for determining the onset of infection (1,7,12,17,21,25).

To determine the primary HCMV infection may be specific IgM are measured especially when the immune state is not known before the pregnancy. Nevertheless, IgM can also be detected in other situation like in recurrent infection and persists for months after primary infection (14). Therefore, the group of women who have HCMV-IgM positive can include women with primary infection acquired before the pregnancy and a few women with recurrent infections (8). IgG avidity assay assists in distinguishing primary infection from past or recurrent infection (1,13,19).

This assay is based on the observation that virus-specific IgG of low avidity is produced during the first months after onset of infection, whereas subsequently a maturation process occurs by which IgG antibody of increasingly higher avidity is generated. IgG antibody of high avidity is detected only in subjects with past or recurrent HCMV infection. Avidity levels are detected as the avidity index, expressing the

percentage of IgG bound to the antigen following treatment with dissociating agents (2). Therefore, serologic diagnosis of primary HCMV infection during pregnancy is documented by either seroconversion (the appearance of HCMV specific IgG antibody in an entirely seronegative woman) or detection of specific IgM antibody associated with low IgG avidity (13).

Women who have a distinguishable level of IgG antibodies without IgM antibodies over pregnancy and a marked ascent of IgG with or without IgM antibodies and with high IgG avidity can be relegated.

Precedent study from Egypt revealed high rates of positive serological tests for HCMV between pregnant women (15).

The aim of the present study was to detect recent infection with HCMV in cohort pregnant Egyptian women in the first trimester by serological detection of specific IgM, IgG and IgG avidity test. The results of serological tests were compared by nested PCR method.

Materials and Methods

This study was performed at the Mansoura Faculty of Medicine, Egypt, between January 2014 and September 2014 on consecutive pregnant women attending outpatient clinics of gynaecology and obstetric for antenatal care. Blood samples were obtained and sera were separated from 120 pregnant women in their first trimester of pregnancy (with mean ± SD duration of pregnancy 10±1.5 weeks). Their mean age was 25.5±5.5 years with mean ± SD gravidity 3.2±1.9 and mean ± SD parity 1.2±1.9. The women were screened for anti-HCMV IgG and IgM antibodies by ELISA (Equipar, Saronno, Italy). The screening tests were essentially performed following manufacturers' instructions. Positive serum for IgG for HCMV was further studied by avidity test.

All women participating in this study gave their informed consent. The Ethical Committee of Mansoura Faculty of Medicine, University, approved the study. PCR was performed for HCMV for all sera obtained from the patients.

Cytomegalovirus IgG avidity

Quantitative determination of HCMV IgG avidity was measured by using the same kit of CMV IgG with the use of eight molar (8 M) urea as a protein-dissociating agent by performing duplicate sets of the routine ELISA for HCMV-specific IgG, one set is washed with buffer containing urea, and the other set is washed with buffer lacking urea. Microplates were washed with phosphate-buffered saline (PBS) and 8 M urea containing PBS solution following incubation of the serum specimens in antigen-coated plates (13). After an eight minutes exposure to the agent at room temperature, the plates were washed and processed. HCMV specific IgG antibody activities in the wells washed with the protein-dissociating agent or PBS only were used to calculate the avidity index (AI), where the AI is expressed as follows: percentage of AI=(absorbance result of CMV per well with urea wash/absorbance result of CMV per well without urea wash) × 100.

It is accounted for, that cut off value of AI >60% is for the most part demonstrative of past or recurrent disease, while cut off value of AI <30% is demonstrating a primary infection (duration 3 months).

Polymerase chain reaction for cytomegalovirus

DNA of HCMV was extracted from a serum sample by QiAamp DNA mini kit (GmbH, Hilden, Germany). For each sample, we prepared the following mixture: buffer (2.5 μL), deoxynucleoside triphosphates (2.5 μL), and primer P1 (1.0 μL), primer P2 (1.0 μL), Taq DNA polymerase (0.3 μL), distilled water (7.7 μL), extracted DNA solution (10 μL), and overlaid mineral oil (30 μL). The PCR for detection of major immediate-early gene MIE region was performed as described (24). The nested primers of morphological transforming region II (mtr II), CMTR 1-5′-CTG TCG GTG ATG GTC TCT TC-3′ and CMTR 2-5′-CCC GAC ACG CGG AAA AGA AA-3′ for the first round and CMTR 3-5′-TCT CTG GTC CTG ATC GTC TT-3′ and CMTR 4-5′-GTG ACC TAC CAA CGT AGG TT-3′ for the second round generated 234-base pair (bp) and 168-bp products, respectively.

Amplification program included initial denaturation at 94°C for 1 minute, followed by 30 cycles of 94, 55 and 72°C for 1 minute each in an automated thermal cycler. Following PCR, the amplicon (168 bp) for HCMV was resolved on a 1.5% agarose gel, visualized using ethidium bromide (0.5 μg/mL) under ultraviolet illumination.

Results

Seventy five women were positive for HCMV IgG (62.5%) and eighteen were positive for IgM (15%). IgG avidity results showed that low avidity IgG, high avidity IgG and intermediate avidity represented 25%, 16.7% and 20.8%, respectively of the total number of the patients. PCR for HCMV was positive in 8.3% of pregnant women (Table 1).

Eighteen pregnant women were positive for IgM, however, only 6 of them (33.3%) were associated with low avidity IgG suggesting recent infection. Nine positive IgM results were associated with high IgG avidity (50%) and 3 positive IgM samples were associated with intermediate avidity IgG results (16.7%). Among pregnant women with negative IgM and positive IgG (n=57), isolated, low avidity IgG was found in 24 patients (42.1%), intermediate avidity IgG avidity in 22 patients (38.6%) and high IgG avidity in 11 patients (19.3%) (Table 2).

We studied pregnant women with nested PCR to confirm serological test results to detect recent HCMV infection. One woman with isolated low avidity IgG was positive by PCR (10%) and 23 were negative. All women with positive IgM and high avidity IgG were negative by PCR. Three women with isolated intermediate avidity IgG were positive by PCR (Table 3). No women IgM positive and IgG negative for HCMV were detected in our cases.

Discussion and Conclusions

Cytomegalovirus infection during the pregnancy can lead to serious sequels for the foetus, resulting in congenital malformations or abortions. Clinical diagnosis is not applicable as the infection usually asymptomatic. Laboratory diagnosis is the milestone for this infection. Previously, laboratories depend mainly on detection of specific IgM for CMV, which has many limitations due to reactivation of HCMV in various conditions. In the present report determination of IgG avidity by in house ELISA method has proven to be sensitive and specific in association with IgM.

In the present study IgM for HCMV was positive in 15%, while IgG was positive in 62.5% among pregnant women.

Results differ in studies according to the used serological method for screening and according to the geographical locations of the study. In Egypt, a recent study reported the prevalence of IgG to be 100% and 7.3% for IgM. All samples with positive IgM in this study had a high AI (15).

The difference in rates can be attributed to the difference of the number of included subjects in the study and the duration of pregnancy. On the other hand, we could not find study about the comparison of the avidity IgG with PCR as a diagnostic tool for recent infection.

Routine serological diagnosis of HCMV provides a rapid screening tool for diagnosis of HCMV. However, there are limited sensitivity and specificity of serological diagnosis depending on the test used.

In this study, 15% of the pregnant women in the first trimester of pregnancy had HCMV specific IgM antibodies, suggesting an acute

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infection that requires prompt therapeutic intervention. Moreover, 62.5% of women had positive IgG. Usually detection of specific IgM for HCMV is not a sensitive indicator of recent infection. Moreover, false positive IgM antibody test results have been reported associated with autoimmune pregnancy complications previously (24). In such cases, the diagnosis of primary infection with HCMV in first trimester of pregnancy can be accurately defined by determination of avidity IgG test.

On avidity testing, 33.3% of IgM positive women had low avidity IgG antibodies indicating a recent HCMV infection in these women. It's worth noticing that positive IgM was reported in 50% of our patients with high avidity IgG indicating non primary infection with no indication for any intervention therapy for those patients.

The apparent inability in detecting acute infection status by IgM serology may be due to the fact that IgM antibodies can persist for months or even years following the acute phase of an infection in some individuals and can be determined in situations of reactivation or reinfection with a different strain of HCMV; therefore the presence of IgM antibodies is not always an indication of a recent infection (5).

In sera with low or intermediate avidity antibodies and negative IgM antibodies, IgG avidity test was potentially misleading, if used alone. In this study, 23 out of 24 IgM-negative women with low avidity antibodies and 19 out of 22 IgM-negative women with intermediate avidity were confirmed negative for HCMV DNA on PCR analysis. Similar results have been reported in previous study (28).

It was previously reported high-avidity antibodies in 92.4% of the

Table 1. Demographic, clinical and serological results for cytomegalovirus in pregnant women (n=120).

Demographic, clinical and serological findings	Studied cases (n=120)
Age (years), mean±SD	25.5±5.5
Residence (%)	
Rural	60 (50)
Urban	60 (50)
Duration of pregnancy, mean weeks±SD	10±1.5
Parity	
Mean±SD	1.2±1.9
Range	0-3
Gravidity, mean±SD	3.2±1.9
Cytomegalovirus	
Specific IgG, n. (%)	75 (62.5)
Specific IgM, n. (%)	18 (15)
PCR, n. (%)	10 (8.3)
Avidity, n. (%)	
Low IgG avidity (<30%)	30 (25)
Intermediate IgG avidity (30-60%)	25 (20.8)
High IgG avidity (>60%)	20 (16.7)

SD, standard deviation.

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Table 2. The results of anti-cytomegalovirus IgM regarding positive IgG avidity results.

Positive IgG avidity (n=75)	Anti-cytomegalovirus IgM	
	Positive (n=18)	Negative (n=57)
Low IgG avidity	6 (33.3%)	24 (42.1%)
Intermediate IgG avidity	3 (16.7%)	22 (38.6 %)
High IgG avidity	9 (50%)	11 (19.3%)

Table 3. Comparison of serological tests with polymerase chain reaction for cytomegalovirus DNA.

Cytomegalovirus serological tests	Polymerase chain reaction, n. (%)	
	Positive (n=10)	Negative (n=110)
IgM positive+intermediate avidity (n=3)	0 (0)	3 (2.7)
IgM positive+high avidity IgG (n=9)	0 (0)	9 (8.2)
IgM negative+low IgG avidity (n=24)	1 (10)	23 (20.9)
IgM negative+intermediate IgG avidity (n=22)	3 (30)	19 (17.3)
IgM positive+low avidity (n=6)	6 (60)	0 (0)
IgM negative+high avidity (n=11)	0 (0)	11 (10)
IgM negative+IgG negative (n=45)	0 (0)	45 (40.9)

IgM-positive serum samples from pregnant women during the first 16 weeks of gestation (3). It is known that the maturation of the IgG response varies considerably between individuals and low or intermediate avidity antibodies may persist for months up to more than 1 year (18). If an avidity test result is used without the presence of IgM this will lead to misinterpretation as an acute infection.

Our findings validate that the use of combined use of IgM and avidity test represents a confirmatory method, most useful if low avidity antibodies are detected in IgM-positive women and also in IgM positive women with high avidity antibodies.

Previous reports have found that PCR can predict the presence of HCMV DNA in blood specimens (9,25). Depending on this finding, the presence of HCMV DNA in the maternal blood usually gives information on the status of infection. However, PCR findings of other report suggest that CMV may or may not be detected in maternal blood in pregnant women undergoing primary infection at the time of diagnosis (17).

In this study, all the IgM-positive women with high-avidity antibodies and women with IgM positive and intermediate avidity antibodies were negative for HCMV DNA on PCR analysis, confirming the high sensitivity and specificity of the avidity test for detecting recent HCMV infection in early pregnancy.

Depending solely on detecting HCMV specific IgM antibodies and/or by detecting a threefold increase in IgG antibodies in follow up samples in pregnant women during the first trimester may result in unnecessary interventions in pregnant women or delay in diagnosis of such stressing condition.

Using PCR analysis to detect HCMV DNA to confirm the recent infection appears as a useful adjuvant diagnostic tool. So, with the use of combined IgM and avidity IgG tests, the use of PCR can be applied to pregnant women with IgM antibodies with the presence of low avidity antibody results or presence of isolated low or intermediate avidity IgG.

References

1. Barbi M, Binda S, Caroppo S, et al. Multicity Italian study of congenital cytomegalovirus infection. Pediatr Infect Dis J 2006;25:156-9.
2. Carlson A, Norwitz ER, Stiller RJ. Cytomegalovirus infection in pregnancy: should all women be screened? Rev Obstet Gynecol 2010;3:172-9.
3. Cavlek TV, Sternak SL, Galinovic G. Value of IgG avidity in cytomegalovirus infection diagnosis in pregnant women and newborn infants. MEJAD6 2008;38:1-2.
4. Colugnat FAB, Staras SAS, Dollard SC, et al. Incidence of cytomegalovirus infection among the general population and pregnant women in the United States. BMC Infect Dis 2007;7:71-81.
5. De Carolis S. Santucci S. Botta A. et al. False-positive IgM for CMV in pregnant women with autoimmune disease: a novel prognostic factor for poor pregnancy outcome. Lupus 2010;19:844-9.
6. Demmler GJ. Infectious Diseases Society of America and Centers for Disease Control: summary of a workshop on surveillance for congenital cytomegalovirus disease. Rev Infect Dis 1991;13:315-29.
7. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol 2007;17:355-63.
8. Drew WL. Diagnosis of cytomegalovirus infection. Rev Infect Dis 1988;10:468-76.
9. Duff P. Diagnosis and management of CMV infection in pregnancy. Perinatology 2010;1:1-6.
10. Forbes BA. Acquisition of cytomegalovirus infections: an update. Clin Microbiol Rev 1989;2:204-16.
11. Gambarotto K, Ranger-Rogez S, Aubard Y, et al. Primary cytomegalovirus infection and pregnant women: epidemiological study on 1100 women at Limoges. Pathol Biol 1997;45:453-61.
12. Gaytant MA, Steegers EAP, Semmekrot BA, et al. Congenital cytomegalovirus infection: review of the epidemiology and outcome. Obstet Gynecol Surv 2002;57:245-56.
13. Grangeot-Keros L, Mayaux MJ, Lebon P, et al. Value of cytomegalovirus (CMV) IgG avidity index for the diagnosis of primary CMV infection in pregnant women. J Infect Dis 1997;175:944-6.
14. Griffiths PD, Stagno S, Pass RF, et al. Infection with cytomegalovirus during pregnancy: specific IgM antibodies as a marker of recent primary infection. J Infect Dis 1982;145:647-53.
15. Kamel N, Metwally L, Gomaa N, et al. Primary cytomegalovirus infection in pregnant Egyptian women confirmed by cytomegalovirus IgG avidity testing. Med Princ Pract 2014;23:29-33.
16. Krech Y. Complement fixing antibodies against CMV in different parts of the world. Bull WHO 1973;49:103-6.
17. Lazzarotto T, Guerra B, Lanari M, et al. New advances in the diagnosis of congenital cytomegalovirus infection. J Clin Virol 2008;41:192-7.
18. Lazzarotto T, Varani S, Spezzacatena P, et al. Delayed acquisition of high-avidity anti-cytomegalovirus antibody is correlated with prolonged antigenemia in solid organ transplant recipients. J Infect Dis 1998;178:1145-9.
19. Liesnard C, Donner C, Brancart F, et al. Prenatal diagnosis of congenital cytomegalovirus infection: prospective study of 237 pregnancies at risk. Obstet Gynecol 2000;95:881-8.
20. Logan S, Tookey P, Peckham C. Antenatal and newborn screening for cytomegalovirus. Report of the working party. London: National Screening Committee; 2000.
21. Naing ZW, Scott GM, Shand A, et al. Congenital cytomegalovirus infection in pregnancy: a review of prevalence, clinical features, diagnosis and prevention. Aust N Z J Obstet Gynaecol 2016;56:9-18.
22. Nelson CT, Demmler GJ. Cytomegalovirus infection in the pregnant mother, fetus, and newborn infant. Clin Perinatol 1997;24:151-60.
23. Onarato IM, Morens DM, Martone WJ, et al. Epidemiology of cytomegaloviral infections; recommendations for prevention and control. Rev Infect Dis 1985;7:479-97.
24. Priya K, Madhavan HN. Use of nested polymerase chain reaction (nPCR) for the detection of cytomegalovirus (CMV) in clinical specimens. Indian J Med Res 2002;115:5-10.
25. Revello MG, Gerna G. Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. Clin Microbiol Rev 2002;15:680-715.
26. Stagno S, Whitley RJ. Herpes virus infections of pregnancy – part I: cytomegalovirus and Epstein-Barr virus infections. N Engl J Med 1985;313:1270-4.
27. Staras SAS, Dollard SC, Radford KW, et al. Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. Clin Infect Dis 2006;43:1143-51.
28. Vilibi -Cavlek T, Ljubin-Sternak S, Vojnovi G, et al. The role of IgG avidity in diagnosis of cytomegalovirus infection in newborns and infants. Coll Antropol 2012;36:297-300.

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