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Value of IgG avidity in cytomegalovirus infection diagnosis in pregnant women and newborn infants

Značaj IgG aviditeta u dijagnostici infekcije citomegalovirusom u trudnica i novorođenčadi

Tatjana Vilibić Čavlek, Sunčanica Ljubin Sternak, Gordana Mlinarić Galinović*

Summary

Aim: To asses the value of IgG avidity in diagnosis of CMV infection in pregnant women and newborn infants.

Methods: During the three-year period (2003-2005) serum samples from 64 pregnant women and 32 infants less than 12 months of age with suspected congenital/perinatal CMV infection were collected. Sera were tested for CMV IgM and IgG using an indirect enzyme-linked immunosorbent assay and IgG avidity using urea as denaturing agent.

Results: Among IgM positive women, 2/26 (7.6%) showed an increase of IgG avidity index (AI) from intermediate to high AI in paired sera samples indicating recent primary infection and 24/26 (92.4%) showed high AI indicating past infection. All women with negative IgM antibodies had high AI. In infants less than 12 months old, acute/recent primary CMV infection was documented in 8/12 (66.7%) children with positive IgM and in 10/20 (50.0%) children with negative IgM antibodies. In two children less than three months with high AI, CMV infection was confirmed by virus isolation from urine. One of them showed a decrease of AI from high to low in paired sera samples.

Conclusions: IgG avidity differentiates primary from nonprimary CMV infection in both pregnant women and infants older than three months. In children less than 3 months of age, transplacentally transferred maternal antibodies of high avidity may influence on the serologic test results.

Key words: CMV, IgG avidity, pregnancy, newborn

Sažetak

Cilj rada: Ispitati vrijednost IgG aviditeta u dijagnostici CMV infekcije u trudnica i novorođenčadi.

Metode: Tijekom trogodišnjeg perioda (2003-2005) prikupljeni su uzorci seruma od 64 trudnice, te 32 djece do godinu dana starosti sa suspektnom kongenitalnom/perinatalnom CMV infekcijom. Serumi su testirani na prisustvo CMV IgM i IgG protutijela metodom ELISA, te aviditet IgG protutijela uz primjenu uree.

Rezultati: U skupini trudnica s pozitivnim IgM protutijelima, u njih 2/26 (7,6%) dokazan je porast indeksa IgG aviditeta (AI), od graničnog u visoki AI u parnim uzorcima seruma, što ukazuje na nedavnu primarnu CMV infekciju, dok je 24/26 (92,4%) imalo visok AI (prošla CMV infekcija). Sve trudnice s negativnim IgM protutijelima imale su visok AI. U djece mlađe od 12 mjeseci, akutna/nedavna primarna CMV infekcija dokazana je u 8/12 (66,7%) djece s pozitivnim IgM, te u 10/20 (50,0%) djece s negativnim IgM protutijelima. U dvoje djece mlađe od 3 mjeseca s visokim AI, CMV infekcija je potvrđena izolacijom virusa iz urina. U jednog od njih došlo je do pada AI iz visokog u niski, u parnom uzorku seruma.

Zaključci: Pomoću testa IgG aviditeta moguće je razlučiti primarnu od prošle CMV infekcije u trudnica i djece starije od 3 mjeseca. U djece mlađe od 3 mjeseca, transplacentarno prenesena majčina IgG protutijela visokog aviditeta mogu utjecati na rezultate seroloških pretraga.

Ključne riječi: CMV, IgG aviditet, trudnice, novorođenčad

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* **Zavod za javno zdravstvo republike Hrvatske, Odjel za virologiju** (mr. sc. Tatjana Vilibić-Čavlek, dr. med., Sunčanica Ljubin-Sternak, dr. med., prof. dr. sc. Gordana Mlinarić-Galinović, dr. med.)

Adresa za dopisivanje / *Correspondence address*: mr. sc. Tatjana Vilibić-Čavlek, dr. med., Zavod za javno zdravstvo Republike Hrvatske, Rockefellerova 12, 10000 Zagreb)

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INTRODUCTION

Human cytomegalovirus (CMV) is now the most common cause of viral intrauterine infection.[1] Congenital CMV infection may be the consequence of either a primary or recurrent maternal infection (reactivation of the virus strain causing primary infection or reinfection by a new virus strain).[2] The main risk for clinically significant congenital disease is primary maternal infection during pregnancy.[3] Fetal damage is seen in 10-20% congenitally infected fetuses after primary infection and in less than 1% cases after recurrent infection.[4] It is, therefore, important to differentiate primary from recurrent infection in pregnant females.[1] Serologic diagnosis of primary CMV infection is usually based on the detection of specific IgM antibodies.[5] However, different clinical situations can be related to the presence of IgM antibodies such as the recurrent infection, the convalescent phase of a primary infection, the persistence of IgM or cross-reactive IgM due to other antigens (herpes viruses other than CMV).[6] In the initial phase of acute viral infection, the average IgG population has low avidity. As the immune response matures over several months, IgG avidity becomes and remains high.[7] An IgG avidity assay can serve as a marker for distinguishing primary (low-avidity antibodies) from nonprimary (high-avidity antibodies) CMV infections.[2]

We analyzed the advantage of IgG avidity in diagnosis of CMV infection in pregnant women and children less than 12 months of age with congenital/perinatal infection.

PATIENTS AND METHODS

Patients

During the three-year period (from January 2003 to December 2005) serum samples from 64 pregnant women and 32 infants less than 12 months of age were analyzed at the Croatian National Institute of Public Health for CMV IgM, IgG and IgG avidity. All infants had clinical diagnosis of suspected congenital/perinatal infection (dystoni syndrome, psychomotor retardation, intracerebral calcifications). The sera were divided into three groups: group I – sera of pregnant women selected on the basis of being CMV IgG positive and IgM positive (N = 26) suggesting acute primary CMV infection; group II – sera of pregnant women selected on the basis of being CMV IgG positive and IgM negative (N = 38) indicating past CMV infection; group III – sera from infants less than 12 months of age who were IgG positive and IgM positive or IgG positive and IgM negative (N = 32). The third group was further subdivided into the following age groups: infants <3 months of age (N = 16), infants 3-6 months of age (N = 9) and infants >6-12 months of age (N = 7).

Methods

Serology

Antibodies to CMV were detected by an indirect enzyme-linked immunosorbent assay using commercial diagnostic kits for IgG (ImmunoLISA-CMV IgG quantitative, Orgenics, Israel) and IgM capture assay for IgM (ImmunoLISA-CMV IgM capture, Orgenics, Israel) antibodies. All samples were tested for IgG avidity. The avidity test was performed with urea as denaturing agent using commercial diagnostic kit (Avidity: Anti-CMV Elisa IgG, Euroimmun, Lubeck, Germany). The IgG avidity index (AI) was calculated and expressed as percentage by dividing the extinction of the sample with urea treatment by the extinction of the sample without urea treatment. The interpretation of AI results has been determined as follows: AI < 40% = low avidity antibodies indicating acute primary infection (within 3 months); AI 40-60% = intermediate avidity (recent primary infection between 4 and 6 months); AI > 60% = high avidity antibodies indicating past infection.[2,4].

Isolation and identification of CMV

Five ml of urine specimen were obtained from 6 patients and cultured on MRC-5 cells (ATCC, Manassas, VA, USA). The presence of CMV virus was determined by observation of cytopathic effect (CPE) and identified using CMV immunofluorescence assay (Light Diagnostics™, Temecula, CA).[8]

RESULTS

In group I (IgG positive/IgM positive pregnant females), in 2/26 sera (7.6%) an increase of AI from moderate (first sample) to high (second sample) was demonstrated indicating recent primary infection. All other sera (92.4%) in this group showed high AI. In group II (IgG positive/IgM negative pregnant females), all sera showed high AI (Figure 1).

In infants less than 12 months of age (group III), twelve patients were IgM positive and twenty patients were IgM negative (Table 1). Using IgG avidity, acute primary CMV infection was demonstrated in 14/32 (37.5%) patients; 6/12 (50.0%) with positive IgM and 8/20 (40.0%) with negative IgM antibodies.

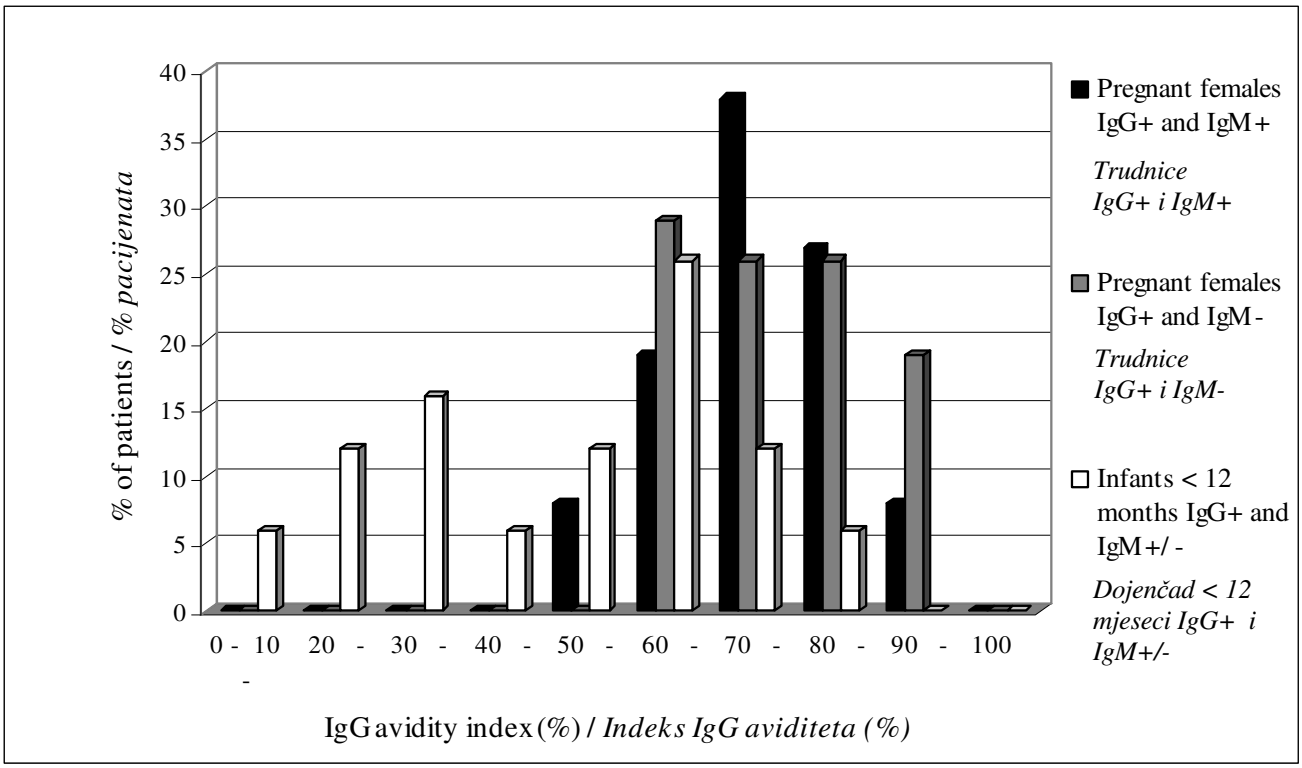

Figure 1. IgG avidity index in 64 pregnant women and 32 infants with suspected CMV infection
Slika 1. IgG aviditet kod 64 trudnice, te 32 dojenčadi sa sumnjom na CMV infekciju

Table 1. Laboratory findings in 32 infants with suspected CMV infection
Tablica 1. Laboratorijski nalazi kod 32 dojenčadi sa sumnjom na CMV infekciju

Patient *Pacijent*	Age *Dob*	ELISA IgM	IgG avidity index *Indeks IgG aviditeta*	Virus isolation *Izolacija virusa*
1	7 days/*dana*	negative/*negativno*	60%	*
2	15 days/*dana*	negative/*negativno*	73%	*
3	20 days/*dana*	negative/*negativno*	84%	*
4	25 days/*dana*	negative/*negativno*	78%	*
5	1 month/*mjesec*	positive/*pozitivno*	69%	*
6	1 month/*mjesec*	negative/*negativno*	80%	*
7	1 month/*mjesec*	negative/*negativno*	64%	*
8	1 month/*mjesec*	positive/*pozitivno*	86%	*
9	1.5 months/*mjeseci*	negative/*negativno*	80%	negative/*negativno*
10	2 months/*mjeseci*	positive/*pozitivno*	18%	*
11	2 months/*mjeseci*	positive/*pozitivno*	20%	*
12	2 months/*mjeseci*	negative/*negativno*	62% / 36%	positive/*pozitivno*
13	2 months/*mjeseci*	negative/*negativno*	60%	*
14	2 months/*mjeseci*	negative/*negativno*	24%	positive/*pozitivno*
15	2 months/*mjeseci*	negative/*negativno*	73%	*

Patient *Pacijent*	Age *Dob*	ELISA IgM	IgG avidity index *Indeks IgG aviditeta*	Virus isolation *Izolacija virusa*
16	2 months/*mjeseci*	negative/*negativno*	36%	*
17	3 months/*mjeseci*	negative/*negativno*	39%	*
18	3 months/*mjeseci*	negative/*negativno*	44%	positive/*pozitivno*
19	3 months/*mjeseci*	negative/*negativno*	86%	positive/*pozitivno*
20	4 months/*mjeseci*	negative/*negativno*	26%	*
21	4 months/*mjeseci*	negative/*negativno*	19%	*
22	4 months/*mjeseci*	negative/*negativno*	49%	*
23	4 months/*mjeseci*	positive/*pozitivno*	51%	*
24	4 months/*mjeseci*	negative/*negativno*	84%	*
25	5 months/*mjeseci*	negative/*negativno*	54%	*
26	7 months/*mjeseci*	positive/*pozitivno*	73%	*
27**	7 months/*mjeseci*	positive/*pozitivno*	51% / 64%	*
28**	7 months/*mjeseci*	positive/*pozitivno*	59% / 69%	*
29	7 months/*mjeseci*	positive/*pozitivno*	37%	*
30	9 months/*mjeseci*	positive/*pozitivno*	19%	*
31	11 months/*mjeseci*	negative/*negativno*	35%	positive/*pozitivno*
32	12 months/*mjeseci*	negative/*negativno*	36%	*

**not done* / nije napravljeno ****identical twins* / jednojajčani blizanci

In 11 children primary infection was confirmed by low AI, and in one (Patient 12) by declining of AI from high to low in paired sera samples. Two IgM positive identical twins (Patients 27 and 28) showed a rise of AI from intermediate to high AI in paired sera samples, indicating recent primary infection. In two children with low AI (Patients 14 and 31) and a child with a decline in AI (Patient 12), CMV was isolated from urine (Table 1).

Recent primary CMV infection (intermediate AI) was detected in 6/32 (18.7%) infants; 4/12 (33.3%) with positive IgM and 2/20 (10.0%) with negative IgM antibodies. However, in one IgM positive child (Patient 18) CMV was isolated from urine.

Two one-month-old (Patients 5 and 8) and one three-month-old (Patient 19) IgM positive babies had high AI. In one of them (Patient 19) CMV was isolated from urine (Table 1).

When the babies were divided into age groups, 4/16 (25.0%) babies less than 3 months showed low AI compared with 3/9 (33.3%) 3-6 months old babies and 4/7 (57.1%) 6-12 months old babies. Four (44.4%) 3-6 month-old babies showed intermediate AI. High AI showed 12/16 (75.0%) babies less than 3 months (Figure 2).

DISCUSSION

The maturation of IgG antibodies has been used as supplementary diagnostic tool for a primary infection identification. This information is particularly important in pregnant women found to be IgM positive to CMV.[1,9]

Authors from Finland have evaluated CMV seroprevalence during pregnancy in three different geographic areas.

The overall CMV IgG seropositivity was 70.7% and the IgM seropositivity was 4.0%. Serologically acute cases defined by low AI represented from 1.0% to 1.7% of the pregnancies.[10] A study conducted in New South Wales, Australia, showed similar results. In this study, a group of 600 pregnant women were tested. Thirty-three women (5.5%) were CMV IgM positive and only 1.2% had a low AI indicating a primary CMV infection.[11]

In our study, using IgG avidity, recent primary infection was confirmed in 2/26 (7.6%) IgM-positive women by increasing AI from moderate to high in paired sera. Twenty-four of twenty-six (92.4%) IgM positive women had high AI which indicated recurrent infection or past CMV infection.

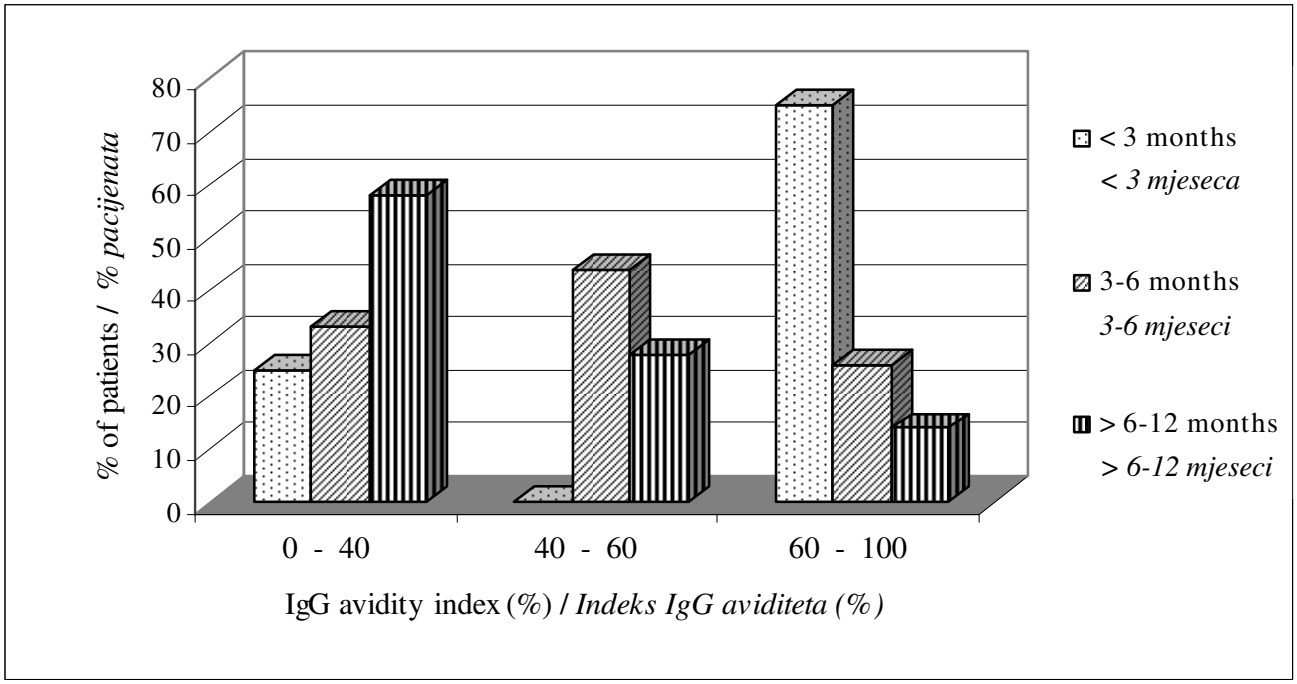

Figure 2. IgG avidity index in 32 infants according to age
Slika 2. IgG aviditet kod 32 dojenčadi prema dobi

IgM antibodies in pregnant women can be found throughout the entire gestational period, and even some months post partum.[7] In addition, the presence of low levels of IgM antibodies may indicate a primary infection initiated some months earlier and possibly prior to pregnancy.[2]

Since maternal IgM antibodies don't cross the placenta, IgM detection in the newborn is likely to be a reliable marker of a primary infection.[2] However, there may be a lack or delay in IgM production in the newborn.[6] Using the IgG avidity, we confirmed acute/recent primary infection in 8/12 (66.7%) IgM positive and 10/20 (50.0%) IgM negative babies. The IgM positive babies older than 3 months had IgM antibodies almost certainly due to primary CMV infection. Eighty-seven point five of these children had AI levels indicating acute or recent primary infection. Some children less than three months (2/19; 10.5%) had high AI, despite primary infection due to transplacentally transferred maternal antibodies of high avidity. Our results are in accordance with results reported in literature. One study was performed in a group of 29 children 3-12 months old with CMV IgM antibodies. Sixteen of the 29 (55.0%) infants had AI levels indicating recent primary infection. When this group was divided into age groups, it appeared that the results for the up to 3-month-old group could be influenced by maternal antibodies (60% of children showed intermediate or high AI).[12]

From the obtained results, it would appear that the IgG avidity differentiates primary from recurrent infections, persistent or false-positive IgM results in both pregnant women and infants older than three months. In children less than 3 months of age, transplacentally transferred high avidity maternal antibodies may have influence on the serologic test results. Therefore, congenital CMV infection should be confirmed by direct virologic methods such as virus isolation or polymerase-chain reaction.

References

1. Bodeus M, Goubau P. Predictive value of maternal IgG avidity for congenital cytomegalovirus infection. J Clin Virol. 1999;12:3-8.
2. 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.
3. Daiminger A, Bader U, Enders G. Pre- and periconceptional primary cytomegalovirus infection: risk of vertical transmission and congenital disease. BJOG 2005;112:166-72.
4. Dangel V, Bader U, Enders G. Improvement of cytomegalovirus avidity test by adjusting the concentration of CMV-specific IgG in test samples. J Clin Virol. 2006;35:303-9.
5. Mace M, Sissoeff L, Rudent A, Grangeot-Keros L. A serological testing algorithm for the diagnosis of primary CMV infection in pregnant women. Prenat Diagn. 2004;24:861-3.

6. Hodinka RL. Human cytomegalovirus. In: Murray PR, Baron EJ, Jorgensen J, Pfaller MA, Yolken EH (eds). Manual of clinical microbiology. 8th ed., Washington: ASM Press, 2003, str. 1304-18.
7. Hermann KL, Erdman DD. Diagnosis by serologic assay. In: Lennette EH, Lennette DA, Lennette ET. Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections. 7th ed. Washington: American Public Health Association, 1996, str. 121-38.
8. Landry ML, Hsiung GD. Primary isolation of viruses. U: Specter S, Lancz G, (ur). Clinical virology manual. New York: Elsevier Science Publishing Company, 1986, str. 31-151.
9. Lazzarotto T, Gabrielli L, Lanari M, et al. Congenital cytomegalovirus infection: recent advances in the diagnosis of maternal infection. Hum Immunol. 2004; 65:410-5.
10. Mustakangas P, Sarna S, Ammala P, Muttilainen M, Koskela P, Koskiniemi M. Human cytomegalovirus seroprevalence in three socioeconomically different urban areas during the first trimester: a population-based cohort study. Int J Epidemiol. 2000; 29:587-91.
11. Munro SC, Hall B, Whybin LR, et al. Diagnosis and screening for cytomegalovirus infection in pregnant women. J Clin Microbiol. 2005;43:4713-8.
12. Blackburn NK, Besselaar TG, Schaub BD, O'Connell KF. Differentiation of primary cytomegalovirus infection from reactivation using the urea denaturation test for measuring antibody avidity. J Med Virol. 1991; 33:6-9.