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Short communication

The Role of IgG Avidity in Diagnosis of Cytomegalovirus Infection in Newborns and Infants

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A B S T R A C T

To evaluate the value of IgG avidity in diagnosis of congenital cytomegalovirus (CMV) infection in newborns and infants we collected serum samples from 40 infants under 12 months of age with suspected congenital CMV infection. Sera were tested for IgM, IgG and IgG avidity. For 25 of them, virus isolation and/or polymerase chain reaction (PCR) on urine specimens were performed. Thirteen (32.5%) patients showed the presence of CMV IgM antibodies, 3 (7.5%) had equivocal IgM result, and 24 (60.0%) patients had IgG antibodies only. Using IgG avidity, CMV infection (low avidity index-AI) was documented in 61.5% IgM positive and 54.2% IgM negative patients. Eight of nine (88.8%) IgM positive patients were positive either on virus isolation or PCR. In IgM negative patients, 46.6% urine cultures were positive for CMV and 66.6% were PCR positive. According to age, IgG avidity demonstrated acute/recent primary CMV infection in 58.8% patients younger than three months compared with 91.7% and 81.8% in 3–6 and 6–12 months old babies, respectively. In conclusion, IgG avidity is useful in diagnosis of CMV infection either in IgM positive or IgM negative children older than 3 months of age. In infants less than 3 months, transplacentally derived maternal IgG antibodies of high avidity influence on the IgG avidity result. In these children, CMV infection should be confirmed by direct virologic methods such as virus isolation or PCR.

Key words: *CMV, diagnosis, IgG avidity, newborns, infants*

Introduction

Cytomegalovirus (CMV) is the most common cause of congenital viral infection in developed countries occurring with an incidence of approximately 1% of all live births[1]. Congenital CMV infection may be the consequence of primary maternal infection, reactivation or reinfection with a new strain in seropositive mother[2]. Transmission of CMV from mother to fetus can occur throughout the entire gestation period, but infection during the first trimester of pregnancy has been associated with a higher incidence of fetal damage[3,4]. Perinatal infection can result from contact with genital secretions during delivery or later in infancy through breast milk[2,5,6].

Determination of IgG avidity could help in distinguishing primary from past CMV infection[7–9]. The term IgG avidity indicates the functional affinity of the IgG class antibody, *i.e.* the strength with which the IgG binds to antigen[1,9]. During the first weeks of primary infection, IgG antibodies show a low avidity for the antigen. The antibody response progressively matures during several months, and afterwards, IgG avidity remains high[10,11]. Full maturation of the IgG antibodies occurs approximately 3–5 months after primary infection in immunocompetent subjects[7,8]. Different studies have shown that the measurement of IgG avidity is a reliable procedure to identify primary CMV infection in pregnant women[7,9,12–15].

The aim of this study was to evaluate the value of IgG avidity in diagnosis of congenital CMV infection in newborns and infants.

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Patients and Methods

The study was conducted as a part of the project »Viral infections of the respiratory tract«, approved by the Ethic Committees of Croatian National Institute of Public Health, Zagreb University Children's Hospital, and University Hospital for Infectious Diseases.

Patients

Between January 2006 and December 2008, serum samples from 40 infants under 12 months of age with clinically suspected congenital CMV infection (development delay, neuromuscular disorders, microcephaly, hearing impairment, intracerebral calcifications) were tested for CMV IgM, IgG and IgG avidity. For 25 of them, urine specimens were obtained and virus isolation and/or polymerase chain reaction (PCR) were performed.

Serology

Anti-CMV IgM and IgG antibodies were detected using commercial enzyme-linked immunosorbent assay (ETI--Cytok M/ETI-Cytok G; Dia Sorin, Saluggia, Italy). The determination of IgG avidity was carried out with urea as denaturing agent using commercial diagnostic assay (Avidity: Anti-CMV Elisa IgG, Euroimmun, Lubeck, Germany). The IgG avidity index (AI) was calculated and expressed as percentage using the extinction values with and without urea treatment. The interpretation of AI results was determinated as follows: AI<40% = low avidity antibodies indicating primary CMV infection; AI 40–60% = moderate avidity; AI>60% = high avidity antibodies indicating past CMV infection.

Isolation and identification of CMV

Five ml of urine specimen was cultured on MRC-5 cells (ATCC, Manassas, VA; USA). The presence of CMV was detected by observation of cytopathic effect and identified using CMV immunofluorescence assay (Light Diagnostics™, Temecula, CA).

Molecular diagnostic

Nucleic acids were isolated from urine using a spin column kit (QIAamp DNA Mini Kit; QIAGEN GmbH, Hilden). Qualitative real-time PCR was applied to the detection of CMV DNA using a TaqMan Universal PCR Master Mix kit (Applied Biosystems). To rule out the presence of PCR inhibitors in individual patient specimens, an internal control kit (TaqMan Exogenous Internal Positive Control Reagents; Applied Biosystems) was used. The amplification and detection were performed with a 7500 Real Time PCR System machine (Applied Biosystems). Each tube contained a 25 μL reaction mix which included 2.5 μL of isolated DNA, 0.9 μM forward primer (5'-CGCTCACATGCAAGAGTTAATCTTT-3'), 0.9 μM reverse primer (5'-AACTCGGTAAGTCTGTTGACA-TGTATG-3') and 0.25 μM probe (5'-FAM-CTCTATCT-GACATACACAAGTAAATCCACGTCCCA-TAMRA-3'). The reaction mixes were exposed to a 2-min, 50°C – UNG enzyme activation, a 10-min of denaturation and DNA polymerase activation at 95°C, and then 45 cycles consisting of 95°C for 15 s and 60°C for 1 min.

Results

Of the 40 sera tested, 13 (32.5%) showed the presence of CMV IgM antibodies out of which 8 (61.5%) had low AI, 3 (23.1%) had moderate AI and 2 patients (15.4%) had high AI. Eight of nine (88.8%) CMV IgM positive patients were positive either on virus isolation or PCR. Three patients (7.5%) showed equivocal IgM results and 24 patients (60.0%) showed the presence of CMV IgG antibodies only. In the group with negative IgM antibodies, thirteen (54.2%) patients had low AI, 5 (20.8%) had moderate AI and 6 patients (25.0%) had high AI. In IgM negative patients, 7 of the 15 (46.6%) cultures were positive for CMV and 8 of 12 (66.6%) urine specimens were PCR positive. In one patient with equivocal IgM results AI was low, and in two patients AI was high. Two of them were positive on both isolation and PCR (Table 1).

IgG avidity demonstrated acute/recent primary CMV infection in 58.8% patients younger than three months (35.2% showed low AI and 23.6% showed moderate AI). In contrast, in 3–6 months and 6–12 months age groups, the percentage of children with primary CMV infection who showed low or moderate AI was higher (91.7% and 81.8%, respectively). Two twelve-month-old infants with high AI were nonidentical twins (one of them showed no IgM antibodies, and the other showed equivocal IgM result) (Table 2).

Results of virus isolation and PCR in 10 patients with congenital CMV infection who showed high AI are shown in Table 3. Two patients had positive IgM, two had equivocal IgM and six patients had negative IgM antibodies. In 2 infants, CMV was isolated from urine and in 4 infants CMV genome was detected in urine using PCR. Three in-

TABLE 1
IgG AVIDITY INDEX IN 40 INFANTS WITH CONGENITAL CMV INFECTION

CMV IgM	N(%)	LOW AI N(%)	Moderate AI N(%)	High AI N(%)	Virus isolation Positive/tested N(%)	PCR Positive/tested N(%)
Positive	13 (32.5)	8 (61.5)	3 (23.1)	2 (15.4)	4/5 (80.0)	4/4 (100)
Equivocal*	3 (7.5)	1 (33.3)	0 (0)	2 (66.6)	2/2 (100)	2/2 (100)
Negative	24 (60.0)	13 (54.2)	5 (20.8)	6 (25.0)	7/15 (46.6)	8/12 (66.6)

* Samples with absorbance values ranging within ± 10% of the cut-off value

TABLE 2
IgG AVIDITY INDEX IN 40 INFANTS WITH CONGENITAL CMV INFECTION ACCORDING TO AGE

Age	N(%)	Low AI N(%)	Moderate AI N(%)	High AI N(%)
<3 months	17 (42.5)	6 (35.2)	4 (23.6)	7 (41.2)
3–6 months	12 (30.0)	10 (83.4)	1 (8.3)	1 (8.3)
6–12 months	11 (27.5)	6 (54.5)	3 (27.3)	2 (18.2)

fants were positive on both isolation and PCR. In only one patient (No 3) results of virus isolation and PCR were negative; however declining of AI from high to low in paired sera samples indicated recent CMV infection.

Discussion and Conclusion

The anti-CMV IgG avidity is often used test for serologic confirmation of primary CMV infection, especially in patients with detectable IgM antibodies. Since maternal IgM antibodies do not cross the placenta, detection of IgM antibodies in the newborn indicates primary CMV infection[2]. However, some congenitally infected infants do not produce IgM antibodies in response to intrauterine infection, or IgM antibody production may be delayed[16]. Revello et al.[2] detected IgM antibodies in 70% of congenitally infected babies. Melish and Hanshaw[17] found that 50% of culture proven CMV infected newborns had IgM antibodies while Schlesinger et al.[18] found that only 2 of 13 (15%) newborns with positive urine samples detected by PCR were IgM positive. Our results also indicate a lack of sensitivity of the newborn IgM status for the diagnosis of congenital CMV infection. We demonstrated anti-CMV IgM antibodies in 32.5% patients. Using IgG avidity, acute or recent CMV infection was demonstrated in 80% of them.

In this study, 60% of patients had no detectable IgM antibodies, while acute CMV infection (low AI) was documented in 54.2% of them. Fifteen (62.5%) urine specimens from IgM negative patients were cultured for CMV and/or PCR; 46.6% of cultures were positive and 66.6% samples were PCR positive. Because determination of IgM antibodies is still considered reasonable for diagnosis of CMV infection in congenitally infected babies, these results emphasizes the importance of IgG avidity testing in IgM positive as well as in IgM negative patients with suspected CMV infection. The presence of low AI in a newborn can be considered a marker of maternal seroconversion in the second or third trimester of gestation and, as a consequence, an indicator of congenital CMV infection. In children older than six months, low AI indicates that primary CMV infection occurred most likely perinataly or postnataly. However, in congenitaly infected babies, IgG avidity maturation may be delayed (up to 2 years) and therefore, low AI could not rule out congenital CMV infection in these children[19].

Interestingly, we demonstrated primary CMV infection in 10 babies despite high AI. In all but one patient, virus isolation and/or PCR confirmed diagnosis of CMV infection. Seven patients (70%) were under 3 months of age and high avidity antibodies were transplacentally derived. In two patients (one who was positive by PCR also), a decline from high to low AI in paired sera confirmed a primary CMV infection. In twelve-month-old nonidentical twins with CMV infection who were PCR positive IgG avidity increased and reached high AI at the time of testing. Blackburn et al.[20] showed similar results. They tested a group of twenty-nine 3–12 month old babies who were CMV IgM and IgG positive. Sixteen of them (55%) showed low AI indicating primary CMV infection. When the babies were subdivided into age groups, six of the ten 3-month-old babies (60%) had moderate to high avidity compared with two of the nine 6–12 months old babies.

In conclusion, the results of this study showed that determination of IgG avidity could be used as a marker for diagnosis of CMV infection both in IgM positive and IgM negative children older than 3 months of age. In infants younger than 3 months, transplacentally derived

TABLE 3
RESULTS OF PCR AND VIRUS ISOLATION IN 10 PATIENTS WITH CONGENITAL CMV INFECTION WITH HIGH IgG AVIDITY INDEX

Patient	Age	CMV IgM	IgG avidity index	PCR	Virus isolation
1	1 mo	equivocal	high	positive	positive
2	2 mo	negative	high/low	NT	positive
3	2 mo	negative	high/low	negative	negative
4	2 mo	negative	high	positive	negative
5	2.5 mo	positive	high	positive	negative
6	3 mo	positive	high	NT	positive
7	3 mo	negative	high	positive	negative
8	5 mo	negative	high	positive	positive
9*	12 mo	equivocal	high	positive	positive
10*	12 mo	negative	high	positive	negative

* nonidentical twins, NT – not tested

maternal IgG antibodies of high avidity influence on IgG avidity result and CMV infection should be confirmed by direct virologic methods such as virus isolation or PCR. However, paired serum samples taken several weeks later could help in the diagnosis of congenital CMV infection (decline of maternal antibodies and appearance of low avidity IgG antibodies) in these children.

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ULOGA IgG AVIDITETA U DIJAGNOSTICI INFEKCIJE CITOMEGALOVIRUSOM U NOVOROĐENČADI I DOJENČADI

SAŽETAK

Cilj rada bio je odrediti ulogu testa IgG aviditeta u u dijagnostici kongenitalne infekcije citomegalovirusom (CMV) u novorođenčadi i dojenčadi. Prikupljeni su uzorci seruma 40 djece mlađe od godinu dana sa suspektom CMV infekcijom i testirani na prisustvo CMV IgM i IgG protutijela te aviditet IgG protutijela. U 25 djece također je učinjena izolacija virusa i/ili lančana reakcija polimeraze (PCR) iz urina. Trinaest (32,5%) bolesnika imalo je prisutna CMV IgM protutijela, 3 (7,5%) bolesnika imalo je graničan IgM nalaz, dok je 24 (60,0%) bolesnika imalo samo CMV IgG protutijela. Pomoću IgG aviditeta, akutna CMV infekcija (nizak indeks aviditeta-AI) dokazana je u 61,5% bolesnika s pozitivnim IgM protutijelima te 54,2% bolesnika s negativnim IgM protutijelima. Osam od 9 (88,8%) IgM pozitivnih bolesnika imalo je pozitivan nalaz izolacije i/ili PCR. U IgM negativnih bolesnika, virus je izoliran u njih 46,6% dok je 66,6% bilo PCR pozitivno. S obzirom na dob, testom IgG aviditeta dokazana je primarna CMV infekcija u 58,8% djece mlađe od tri mjeseca, 91,7% djece u dobi od 3–6 mjeseca te 81,8% djece u dobi od 6–12 mjeseci. U zaključku, test IgG aviditeta može pomoći u dijagnostici CMV infekcije u djece starije od 3 mjeseca s pozitivnim kao i negativnim IgM protutijelima. U djece mlađe od 3 mjeseca, transplacentarno prenesena majčina protutijela visokog IgG aviditeta utječu na rezultat AI. Stoga u toj dobnoj skupini CMV infekciju treba potvrditi izravnim virološkim metodama kao što je izolacija virusa ili PCR.