

## ORIGINAL ARTICLE

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# Relationship of IgG Avidity Index and IgM Levels for the Differential Diagnosis of Primary from Recurrent Cytomegalovirus Infections

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## ABSTRACT

Since the incidence of symptomatic congenital cytomegalovirus infection is low (~0.05%) and risk factors are not well defined, it is difficult to develop strategies for prevention. The aim of this study was to recognise the utility of specific Immunoglobulin G avidity analysis for distinguishing primary infection from past/recurrent infection.

Sera from 50 women with cytomegalovirus specific Immunoglobulin M antibodies without proven seroconversion and infants born to these women were tested for presence of Immunoglobulin M antibodies by commercial enzyme immunoassay.

For cytomegalovirus specific immunoglobulin G avidity, sera were measured by commercial kit according to manufacturer's recommendations.

Among 50 sera from mothers, 26 showed the presence of Immunoglobulin M antibodies out of which 15 had low avidity antibodies. Out of 50 sera from children, 18 showed the presence of Immunoglobulin M antibodies. Out of these 18 sera from children, 12 were symptomatic, which all showed the presence of low avidity antibodies.

The results showed that an avidity index <40% and presence of Immunoglobulin M antibodies is highly suggestive of a recent primary infection.

**Key words:** Avidity index; Cytomegalovirus; Congenital; IgM antibody

## INTRODUCTION

Cytomegalovirus (CMV) is the most common intrauterine viral infection in humans, occurring in 0.4%-2.3% of all infants born alive.<sup>1,2</sup> Approximately 50% of females have been found to be seropositive prior to pregnancy giving a 5-15% risk of reactivation during pregnancy and a 0.7-4% risk, depending on socioeconomic status, of primary CMV infection.<sup>3</sup>

Though CMV infection is endemic throughout the world,<sup>4</sup> it is more common in the developing countries, which is predominantly related to the closeness of contacts within these populations. Except for a mononucleosis-like illness in some persons, CMV infection in normal immunocompetent adults is typically clinically silent and chronic with viral shedding continuing for months or longer,<sup>5</sup> but its significance is many times increased when it occurs during pregnancy.<sup>6</sup> Congenital CMV infection is silent at birth in 90% of infants, yet 5-17% of these neonates will develop neurological impairments.<sup>2</sup> Up to 10% of the infants have severe symptoms at birth and the

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survivors invariably suffer severe neurological sequelae. Preconception seroimmunity to CMV provides substantial protection against intrauterine transmission and damaging fetal infections.<sup>7,8</sup> Transmission of CMV to the fetus follows approximately 30-40% of primary maternal infections, whereas < 0.5% of women who are seropositive before pregnancy deliver infected infants.<sup>9,10</sup> In the general population, seroconversion rates of 0.7-4.1% have been observed during pregnancy.<sup>11</sup> It is commonly recognized that primary CMV infections are transmitted more frequently to the fetus and are more likely to cause fetal damage than recurrent infections.<sup>12</sup> It would thus be clinically important to be able to differentiate primary maternal CMV from reactivation infection or reinfection.

CMV immunoglobulin M (IgM) detection is a very sensitive marker for primary infection, but unfortunately, it is not specific.<sup>13</sup> Moreover, IgM demonstration is frequently hampered by the attachment of Rheumatoid factor (RF) of the IgM class (IgM-RF) to specific IgG, leading to positive reaction in the absence of specific IgM. Likewise, detection of increasing CMV IgG levels over time is usually practical only for closely monitored patients for whom pre and post infection sera are readily available. However, it is an unreliable approach for distinguishing primary from nonprimary CMV infection, since most seropositive patients show high IgG levels in the first serum sample collected for testing.<sup>13</sup>

Avidity is defined as the strength with which the IgG attaches to antigen. IgG avidity matures with the length of time following primary infection. IgG produced within the first 3-5 months following primary infection exhibits low avidity, whereas IgG produced months or years later exhibits high avidity.<sup>14</sup> In a recent study, an avidity index (AI) above 65 % during the first trimester of pregnancy could reasonably be considered a good indicator of past CMV infection, whereas in all women with a low AI ( $\leq 50\%$ ), there was a risk of congenital CMV infection.<sup>15</sup> Maternal infections, which have been considered as significant factors in the causation of poor pregnancy outcome, have not been extensively studied so far in India. The purpose of this study was to evaluate the ability of a commercially available ELISA-IgM kit to diagnose congenital CMV infection, to determine if measurement of the CMV AI could also help to both confirm and clarify the clinical significance of IgM antibody to distinguish primary

from non-primary infection and to provide a better definition of the relative roles of primary and recurrent maternal CMV infections in intrauterine transmission.

## MATERIALS AND METHODS

### Study Population

Sera from 50 women without well documented seroconversion and also from 50 infants born to these women were tested for CMV infection. These specimens were stored at  $-20^{\circ}\text{C}$  till tested. A detailed questionnaire was filled up for each one of them regarding their socioeconomic status, geographical area (rural/urban), birth history and clinical signs and symptoms etc.

### CMV IgM ELISA

The CMV IgM levels for these sera were measured using the Cytomegalovirus IgM-ELISA manufactured by Novatec Immunodiagnostica GMBH, which gives the qualitative immunoenzymatic determination of IgM-class antibodies against CMV based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. The assay was performed according to the manufacturer's instructions. The samples were considered positive if the absorbance value was higher than 10% over the cut-off and negative if the absorbance value was lower than 10% below the cut-off. Samples with an absorbance value of 10% above or below the cut-off were considered in the grey zone and a repeat test was done in those cases with a fresh sample 2-4 weeks later. To exclude the possibility of false-positive reactions being produced by rheumatoid factor, serum samples (10 $\mu\text{l}$ ) giving positive reactions were incubated for one hour at  $37^{\circ}\text{C}$  with 500 $\mu\text{l}$  of latex bead suspension containing human IgG.

### CMV-IgG avidity ELISA

The CMV-specific IgG avidity was measured by the Euroimmun Medizinische labordiagnostika AG kit according to the manufacturer's recommendations. 100 $\mu\text{l}$  of controls as well as the patient samples were incubated in duplicate in well each of two different microtiter strips at room temperature (RT) for 30 minutes. After washing, as recommended, 200 $\mu\text{l}$  of urea solution was pipetted into each of the wells of the first strip and 200 $\mu\text{l}$  of phosphate buffer was pipetted into each of the wells of the second strip and incubated at RT for 10 minutes. After washing 100 $\mu\text{l}$  of enzyme

## Avidity index in Cytomegalovirus infection

conjugate (peroxidase-labelled anti-human IgG) was pipetted into each of the wells and incubated for 30 minutes at RT. After washing 100µl of chromogen/substrate solution was pipetted into each of the wells and incubated for 15 minutes at RT protected from direct sunlight. After pipetting 100µl of stop solution into each of the wells photometric measurement was done at 450nm wavelength. The relative avidity index (RAI) was calculated and expressed in percent using the extinction values with and without urea treatment.

$$RAI \text{ in percent} = \frac{\text{Extinction of the sample with urea treatment}}{\text{Extinction of the sample without urea treatment}} \times 100$$

The upper limit of the range of low avidity antibodies (cut-off value), as recommended by EUROIMMUN, is 40% RAI. Values below 40% were considered as an indication of low avidity antibodies, values between 40% and 60% RAI as equivocal, and values above 60% as an indication of high avidity antibodies. If a result was classified as equivocal, a second sample was collected not less than 7 days from the participants and was tested with the first sample.

### RESULTS

Among the 50 women without proven seroconversion from whom sera were collected, the age of majority (70%) were between 20 to 30 years of age. The mean age was 26 years and the mean parity was 3.7. Fifty percent belonged to the low socioeconomic group and their education ranged from illiterate (40%) to higher education (25%). Among the 50 women, 37.5% came from rural background.

IgM antibodies were detected by a commercially available ELISA kit in 26 out of 50 (52%) pregnant women. Of the 26 women who had detectable IgM antibodies by ELISA, 11 (42.3%) delivered symptomatic infants with low AI, whereas of the 19 women with undetectable IgM antibodies, only 1 (5.3%) was considered to be infected. Specific IgM antibodies were detected by ELISA in 12 out of 12 (100%) symptomatic babies with low AI and in only 6 out of 38 (15.8%) uninfected babies.

Among the 50 sera that were obtained from the mothers, 19 were presumed to represent past CMV infection based on the absence of CMV specific IgM and 5 showed equivocal results. Out of these 19 sera, 16 (84.2%) exhibited CMV IgG AI values of >60% and

only 3 sera (15.8%) had AI values of <40%. None of the 5 equivocal sera exhibited AI values of <40%. Of the remaining 26 CMV IgM-positive sera, AI values were broadly distributed; 15 out of 26 sera (57.7%) exhibited low avidity, whereas 4 out of 26 sera (15.4%) exhibited intermediate avidity and 7 out of 26 sera (26.9%) exhibited high avidity. These sera were further used to investigate the relationship between CMV IgG avidity and CMV IgM levels, as represented by the absorbance values using the CMV IgM ELISA from NOVATEC (Table 1). All pregnancy outcomes were known for these 50 women. Twelve babies were symptomatic and had low AI and all 12 were born to women with low AI (<40%). Twelve out of 18 IgM positive babies (66.7%) had AIs less than 40% and none of the babies with undetectable IgM antibodies or equivocal results had low AI (Table 2).

The rate of intrauterine transmission was 66.7% (8 out of 12) in the low-income group and 33.3% in the higher income group. Of the 13 women with low AI who gave birth to symptomatic babies, only 3 (23%) had signs and symptoms compatible with acute CMV infection. This patient was of middle to upper socioeconomic background and presented with mononucleosis like syndrome. Of the 50 infants evaluated, only 13 (26%) had signs and symptoms compatible with congenital CMV infection, which was clinically detected in nursery and all were born to mothers with low AI.

**Table 1. Distribution of CMV IgG avidity values in relation to NOVATEC CMV IgM ELISA absorbance values for 50 mother sera.**

NOVATEC absorbance value	N.	No. of sera with indicated CMV IgG avidity		
		Low	Intermediate	High
>10% over cut off	26	15	4	7
10% above/below cut off	5	0	0	5
<10% below cut off	19	3	0	16

**Table 2. Avidity Index results for 50 infants in relation to the IgM positivity.**

IgM positivity	N.	No. of sera with indicated CMV IgG avidity		
		Low	Intermediate	High
Positive	18	12	4	2
Negative	30	0	0	30
Equivocal	2	0	0	2

Nine of these 13 (69.2%) symptomatic infants had hepatomegaly and jaundice; 4 (30.8%) of them had evidence of CNS damage. Of these 13 symptomatic babies, 2 (15.4%) died.

## DISCUSSION

The inconspicuous nature of CMV infection in practically all mothers and in the majority of newborns, even in many that later develop sequelae, renders diagnosis difficult. In view of the continuing importance as a cause of morbidity and mortality, CMV presents a significant challenge for those who wish to develop strategies to prevent congenital disease. This study which included pregnant women with different socioeconomic background, supports previous observation<sup>16</sup> that the intensity of exposure to CMV in our community is considerable since 30.8% of women of middle to upper socioeconomic background and 69.2% of those of low socioeconomic background showed the presence of CMV specific IgM antibodies and adverse living conditions, poor hygiene and closeness of contact as factors responsible. As in earlier studies,<sup>17</sup> the majority of women with primary CMV infection remained asymptomatic. The risk of transmission in-utero was somewhat higher among women with detectable IgM antibodies as opposed to those whose tests remained negative as seen in previous studies<sup>[18]</sup>. Serodiagnosis based on virus specific IgM assay still does not seem to be the best in demonstrating congenital CMV infection because there is a risk of missing recent primary infection or conversely of diagnosing as primary a recurrent infection. Moreover, since in a small proportion of women with primary infection, IgM antibodies may be detectable for >16 weeks, a positive result obtained in the early phase of pregnancy would be difficult to interpret particularly in attempting to estimate the risk to the fetus.<sup>16</sup> Therefore, recently the usefulness of CMV specific IgG avidity analysis is recognized for distinguishing primary from past or recurrent infection.<sup>19</sup> To assess the contribution of this method for dating CMV infection in pregnant women, we studied the CMV AI pattern in women with and without specific IgM but lacking well-documented seroconversion. Of the 26 sera that were positive for IgM antibodies, 15 exhibited low CMV IgG avidity, whereas only 3 of the 19 sera that were IgM negative and presumed to represent past infection exhibited low CMV IgG avidity. On the other hand out of the 18 sera

from infants that were positive for IgM antibodies, 12 had low IgG avidity, whereas none of the 30 sera that were negative for IgM antibodies exhibited low IgG avidity. Of the 18 mothers with low avidity antibodies, 13 gave birth to symptomatic babies and 5 were asymptomatic, 3 of them being IgM positive. All the 12 babies showing low avidity antibodies were symptomatic and positive for IgM antibodies. The vast majority CMV IgM positive sera with intermediate/high IgG avidity, presumed to represent either long lasting IgM produced during primary infection many months earlier or IgM produced during reinfection or reactivation. The AI value selected for discriminating low versus high CMV IgG avidity in our kit were consistent with values published by other investigators.<sup>20</sup> As demonstrated by others<sup>21</sup>, our study shows that due to its superior clinical utility for distinguishing primary from nonprimary CMV infection; an IgG avidity assay could be used in combination with ELISA IgM for monitoring pregnant women for primary CMV infection.

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## Avidity index in Cytomegalovirus infection

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