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RESEARCH ARTICLE

The relationship between cytomegalovirus IgM index value and low IgG avidity

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ABSTRACT

Background: Serological markers like anti-CMV IgG, IgM, and IgG avidity are used to diagnose cytomegalovirus (CMV) infection. This study assessed the seroprevalence of CMV antibodies and the predictive value of the CMV IgM index for low IgG avidity.

Materials and methods: A total of 5,666 serum samples collected between January 2017 and June 2021 were analyzed for CMV IgG and IgM using chemiluminescence immunoassay. IgG avidity was tested by enzyme-linked immunosorbent assay in 40 IgG-positive and borderline/positive IgM samples.

Results: Anti-CMV IgM seroprevalence was 4.3%. Low IgG avidity was detected in 2 of 40 cases (5.0%). Receiver operating characteristic (ROC) analysis identified 2.26 as the optimal IgM index cut-off, yielding 100% sensitivity, 86.8% specificity (area under the curve = 0.895, p = 0.06).

Conclusions: High CMV IgM index values may indicate low IgG avidity but are not sufficient alone. Results should always be interpreted alongside avidity testing. Threshold use without confirmation is not recommended.

Keywords: cytomegalovirus, avidity, ROC analysis, sensitivity, specificity

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INTRODUCTION

Cytomegalovirus (CMV) belongs to the genus *cytomegalovirus* in the *β-herpesvirus* subfamily of the *herpesviridae* family [1]. It is widely prevalent across the globe, with anti-CMV IgG seroprevalence ranging from 8.7% to 99.2%, depending on the population studied [2]. While CMV infection is typically asymptomatic in immunocompetent individuals, it can lead to serious complications in immunosuppressed patients, such as those undergoing immunosuppressive therapy, recipients of solid organ or hematopoietic stem cell transplants, and individuals receiving frequent blood transfusions [3]. Importantly, CMV is also the leading cause of congenital viral infection worldwide [4].

Primary CMV infection during pregnancy poses a significant risk to the fetus, with an estimated 40% transmission rate if the mother acquires the infection during gestation. In contrast, the risk is approximately 1% for infants born to mothers with pre-existing immunity [5-7]. Therefore, accurate diagnosis of primary infection or reinfection (with a different CMV strain) during pregnancy is essential for guiding clinical management and prenatal counseling [8].

Several serological markers are utilized to determine CMV infection status, including anti-CMV IgG and IgM antibodies and CMV IgG avidity testing [9]. Commonly used diagnostic techniques include enzyme immunoassay, enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination, and radioimmunoassay for the detection of anti-CMV IgG antibodies [10]. Seroconversion of IgG is a definitive indicator of primary CMV infection [8]. Among the available methods for detecting anti-CMV IgM antibodies, ELISA remains the most frequently employed. However, a positive IgM result may persist for months following acute infection and does not necessarily indicate a recent primary infection. Moreover, the

specificity of IgM assays for detecting primary infection is relatively low due to the risk of false positives [10-12].

Due to the limitations of IgM-based diagnostics, CMV IgG avidity testing is widely implemented as a reliable tool to differentiate primary from non-primary infections and is considered the current "gold standard," particularly during pregnancy [6, 13-17].

The principle of IgG avidity testing is based on the natural maturation of antibodies. Over time, B lymphocytes produce antibodies with stronger binding affinities through a process known as affinity maturation within germinal centers. This leads to increased cumulative binding strength, termed avidity, between multivalent IgG antibodies and their target antigens. Low avidity IgG antibodies are typically detectable during the early months following primary CMV infection, whereas high avidity IgG indicates a past or chronic infection. The avidity index (AI) is defined as the percentage of IgG antibodies that remain bound to their antigen after treatment with denaturing agents. Consequently, IgG avidity testing is widely used as a valuable tool to differentiate between primary and non-primary CMV infections, particularly in prenatal screening settings [5, 16, 18].

In most clinical laboratories, CMV IgG avidity testing is conducted only on samples that are positive for both CMV IgG and IgM to distinguish primary from secondary infections [5]. However, studies have shown that low IgG avidity may also occur in 1%-3% of samples that are IgG positive but IgM negative [8, 17, 19]. The high sensitivity of CMV IgM assays contributes to the likelihood of identifying cases with low IgG avidity [19-21].

This study aimed to determine the seroprevalence of CMV antibodies and evaluate whether the CMV IgM index value could serve as a predictive marker for low IgG avidity, thereby potentially improving the early identification of primary CMV infection.

MATERIALS AND METHODS

This study was designed as a single-center, retrospective analysis. It included patients of all ages whose serum samples were tested for anti-CMV (IgM and IgG) antibodies due to suspected CMV infection across various clinical departments at University of Health Sciences between January 1, 2017, and June 30, 2021. Demographic information, including the date of request, diagnosis, age, and gender, was retrieved from the hospital's electronic medical record system. Patients with insufficient serum volume or inconclusive test results were excluded. In cases with multiple test records, only the first test result was considered. To examine CMV seroprevalence across the lifespan, patients were categorized into eight predefined age groups.

CMV Assay

Serum samples were analyzed using the Architect i2000SR system (Abbott, USA) with commercially available CMV IgG and IgM kits, based on the chemiluminescence immunoassay method. Semi-quantitative detection of anti-CMV IgG and IgM antibodies was performed in accordance with the manufacturer's instructions. Anti-CMV IgG levels equal to or above 6 arbitrary units per milliliter (AU/mL) were considered reactive. For CMV IgM, index values between 0.85 and 0.99 were interpreted as equivocal, while index values equal to or greater than 1.00 were classified as reactive.

CMV IgG Avidity Test

CMV IgG avidity was measured using the Dia.Pro® Diagnostic ELISA kit (Milan, Italy), according to the manufacturer's protocol. The test was applied only to samples that were positive for anti-CMV IgG and equivocal or positive for anti-CMV IgM. The AI was calculated as a percentage to determine the binding strength of IgG antibodies. Results were classified into three categories: low avidity (AI ≤ 40%), moderate avidity (AI between 40% and 60%), and high avidity (AI ≥ 60%). An AI of 40% or lower was accepted as indicative of a recent primary CMV infection.

Statistical Analyses

All statistical analyses were performed using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics for continuous variables were expressed as medians and inter-quartile ranges (IQRs). The Mann-Whitney U test was used for comparing two independent groups, and the Kruskal-Wallis test was applied for comparisons involving more than two groups. Categorical variables were compared using Pearson's chi-square test or Fisher's exact test, where appropriate. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of CMV IgM index values in predicting low IgG avidity. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated based on the optimal threshold identified in the ROC analysis. All statistical results were reported with 95% confidence intervals, and a P value less than 0.05 was considered statistically significant.

RESULTS

CMV IgG and IgM Prevalence

Among a total of 5666 individuals aged between 0 and 97 years (median age: 30 years; IQR: 21-43), the overall anti-CMV IgG seroprevalence was 95.5%. Males constituted 40.6% of the study population. The overall anti-CMV IgM seroprevalence was 4.3%.

CMV IgM positivity was significantly more common in individuals aged ≤ 18 years, with a prevalence of 7.7% in this group ($p < 0.001$). The median age of CMV IgM-positive

Table 1. Socio-demographic characteristics of the study population

Characteristics		Number of tested	No (%) Positive CMV IgG	p-value*	No (%) Positive CMV IgM	p-value *
Gender	Male	2,300	2,166 (94.2)		109 (4.7)	
	Female	3,366	3,247 (96.5)	< 0.001	135 (4.0)	0.190
Total		5,666	5,413 (95.5)		244 (4.3)	
Age group (years)	≤ 18	1,100	996 (90.5)		85 (7.7)	
	19-30	1,833	1,767 (96.4)		76 (4.1)	
	31-40	1,123	1,088 (96.9)		36 (3.2)	
	41-50	584	568 (97.3)	< 0.001	20 (3.4)	< 0.001
	51-60	445	433 (97.3)		14 (3.1)	
	61-70	302	287 (95.0)		7 (2.3)	
	71-80	203	198 (97.5)		6 (3.0)	
	> 80	76	76 (100)		0 (0.0)	
Years	2017	632	601(95.1)		7 (1.1)	
	2018	1,332	1,282 (96.2)		50 (3.8)	
	2019	1,512	1,446 (95.6)	0.060	73 (4.8)	< 0.001
	2020	1,280	1,206 (94.2)		78 (6.1)	
	2021	910	878 (96.5)		36 (4.0)	
Etnicity	Turkish	5,537	5,287 (95.5)	0.230	236 (4.3)	0.280
	Others	129	126 (97.5)		8 (6.2)	

Note. Ig, immunglobulin & *p < 0.05 was considered statistically significant

Table 1. Results of CMV IgM by chemiluminescence immunoassay methods and CMV IgG avidity index

CMV IgM	AI			Total n
	Low n (%)	Intermediate n (%)	High n (%)	
Positive (n = 27)	2 (5.0)	2 (5.0)	23 (57.5)	27
Equivocal (n = 13)	0 (0.0)	1 (2.5)	12 (30.0)	13
Total (n = 40)	2 (5.0)	3 (7.5)	35 (87.5)	40

Note. Percentages are calculated based on the total number of patients (n = 40)

individuals was 23.5 years (IQR: 10-36), while that of CMV IgM-negative individuals was 30 years (IQR: 22-44), which was statistically significant (p < 0.001).

The majority of the study population (97.7%) were Turkish citizens. CMV IgM positivity was 4.3% among Turkish citizens and 6.2% among non-citizens; however, the difference was not statistically significant (p = 0.28) (**Table 1**).

Avidity Test Results

Avidity testing was conducted on 40 serum samples that were both anti-CMV IgG positive and either borderline or positive for anti-CMV IgM. The age of these patients ranged from 0 to 60 years (median: 27.5; IQR: 23-32). Of the 40 individuals, 38 (95%) were female and 2 (5%) were male. Anti-CMV IgM was borderline at 32.5% (13/40) and positive in 67.5% (27/40). Among female patients, 78.9% (30/38) were pregnant.

Low IgG avidity, indicative of recent primary infection, was identified in 2 out of 40 patients (5.0%) (**Table 2**). Notably, no cases with an anti-CMV IgM index value below 2.55 exhibited low avidity.

Anti-CMV IgM index values were categorized into four groups: 0.85-0.99, 1.00-1.99, 2.00-2.99, and ≥ 3.00. The distribution of low, intermediate, and high avidity results across these index value ranges is presented in **Table 3**.

The median anti-CMV IgM index in the low avidity group was 3.41 (IQR: 2.55-4.27), whereas it was 1.10 (IQR: 0.93-1.38) in the high avidity group (p = 0.06).

ROC curve analysis was conducted to evaluate the predictive value of the anti-CMV IgM index for identifying low IgG avidity, using CMV IgG avidity testing as the reference standard. An index value of 2.26 was determined as the optimal cut-off point. At this threshold, sensitivity was 100% (95% CI: 34.2-100), specificity was 86.8% (95% CI:

Table 3. The distribution of low, moderate and high CMV IgG avidity on different CMV IgM index ranges

Igm index range	Total	AI			p-value
		Low	Moderate	High	
	n	n (%)	n (%)	n (%)	
0.85-0.99	13	0 (0.0)	1 (2.5)	12 (30.0)	0.04
1.00-1.99	20	0 (0.0)	1 (2.5)	19 (47.5)	
2.00-2.99	2	1 (2.5)	0 (0.0)	1 (2.5)	
≥ 3.00	5	1 (2.5)	1 (2.5)	3 (7.5)	
Total	40	2 (5.0)	3 (7.5)	35 (87.5)	

Note. Percentages are calculated based on total sample size (n = 40)

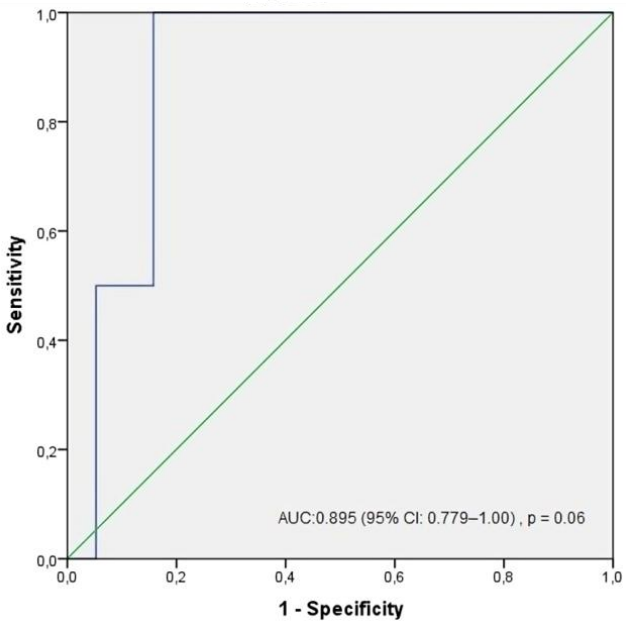

Figure 1. ROC curve of CMV IgM index ratio for predicting low CMV IgG avidity (CI: Confidence interval) (Source: Authors' own elaboration)

72.7-94.2), the PPV was 28.6% (95% CI: 8.2-64.1), and the NPV was 100% (95% CI: 89.6-100). The area under the curve (AUC) was calculated as 0.895 (95% CI: 0.779-1.00; p = 0.06) (**Figure 1**).

DISCUSSION

In this study, we investigated the seroprevalence of CMV antibodies in a large cohort of 5,666 patients and evaluated whether CMV IgM index values could serve as a predictor for low CMV IgG avidity. This study confirmed the very high CMV seroprevalence observed in many populations [2, 22-25], supporting the global burden of CMV infection as also highlighted in previous large-scale studies [26-30].

CMV IgM antibodies can be detected in both primary and non-primary infections, such as reactivation or reinfection with different strains, thereby limiting their specificity for identifying recent infections [5, 8, 19, 27]. The combination of CMV IgM positivity with low IgG avidity is therefore considered a more reliable indicator of recent infection [26]. However, IgG avidity results may vary depending on the timing of sample collection and the commercial test kit used, and intermediate avidity values often present interpretive challenges [18, 28-30]. Despite these limitations, CMV IgG avidity testing remains the gold standard for serological confirmation of primary infection, particularly in pregnant women, where clinical decisions may depend on its results [5, 19].

Previous studies have suggested that high CMV IgM index values may correlate with low IgG avidity, especially in pregnant women with suspected recent infection [8, 9, 31]. For example, the study in [31] reported that an IgM index ≥ 3.0 predicted low avidity with over 90% accuracy. Other studies have proposed various thresholds (e.g., ≥ 1.21, ≥ 2.00, ≥ 4.00, or ≥ 6.00) that may indicate increased risk of congenital CMV transmission.

In our study, only a small proportion of cases showed low IgG avidity, likely reflecting the persistence of IgM positivity or possible false-positive results [32]. Since avidity testing was performed in a limited subgroup of patients with borderline or positive IgM results, the generalizability of our findings is restricted. ROC analysis suggested a potential IgM index threshold for predicting low avidity, but the borderline statistical significance (p = 0.06) and limited sample size reduce its clinical applicability.

Our findings, consistent with prior literature, indicate that anti-CMV IgM index values may be useful for predicting low CMV IgG avidity, and thus for identifying recent primary CMV infection. However, while these findings demonstrate an association between higher IgM index values and low IgG avidity, we emphasize that the IgM index alone is insufficient to confirm primary CMV infection. IgG avidity testing remains essential, particularly in pregnant patients, to ensure diagnostic accuracy. The high NPV observed at the 2.26 index cut-off may support the exclusion of recent infection in selected clinical scenarios, but this index should always be interpreted with caution and confirmed through avidity testing. Nevertheless, the low PPV (28.6%) weakens the discriminatory power of the test, and the limited number of cases further reduces the

statistical strength of the ROC analysis. Although determining assay-specific IgM index thresholds may assist laboratories in interpreting results, these thresholds currently lack international validation and standardization and therefore cannot be recommended for routine clinical application at this time.

Numerous factors can influence CMV IgM index values, including the time elapsed since infection onset, assay sensitivity, test calibration, inter-individual immune response variability, and potential cross-reactivity with other herpesviruses [28-31]. Additionally, CMV reactivation or reinfection with a different strain may elevate IgM levels without indicating a primary infection. These variables highlight the importance of interpreting IgM index values with clinical and laboratory context in mind [8, 19, 27, 32].

This study has some limitations. As a retrospective analysis, it lacked access to clinical outcomes such as pregnancy progression and neonatal data. Furthermore, the sample size for avidity testing was relatively small, which may limit the generalizability of our findings. In addition, the relatively small number of avidity-tested samples and the borderline statistical significance of the ROC curve should be considered when interpreting these findings.

CONCLUSION

Reliable and noninvasive serological tests are essential in the diagnostic evaluation of primary CMV infection. In our study, we observed that higher anti-CMV IgM index values were associated with low IgG avidity, indicating a potential role in identifying recent infections. However, IgM results alone are not definitive and should always be interpreted in conjunction with IgG avidity testing. Therefore, establishing universal or kit-specific threshold values without confirmatory avidity testing is not recommended for routine clinical practice.

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