THE VALUE OF CMV AND RUBELLA IGG AVIDITY TESTS IN THE DIAGNOSIS OF CYTOMEGALOVIRUS (CMV) AND RUBELLA INFECTIONS IN PREGNANT WOMEN

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ABSTRACT
IgG avidity tests, which came on the scene recently with regard to most efficient use of time and early onset to the therapy, are considered in this study, and compared with Enzyme immunoassay (EIA). Serums of a total of 879 women in the first trimester of their gestations who referred to Dicle University, Faculty Hospital, Department of Gynecology and Obstetrics in between the dates of 09 April 2002 – 09 May 2003 were evaluated with the classical method ELISA (Cobas Core II, Roche, USA), in terms of Rubella and HCMV IgG and IgM. Avidity tests were performed with (IgG avidity EIA. Well; Radim, Italy) commercial kits. 4 samples among the 8 that were positive for HCMV IgM and IgG were found to have low AIs (AI<35%), 2 samples to have intermediate AIs (AI 35-45%), and the other 2 to have high AIs (AI >45%). One of the 156 IgG positive serum samples had an intermediate AI (AI 35-45%), and 155 had high AIs. In the Rubella IgG avidity tests, 9 of the 13 IgM and IgG positive samples had low AIs (AI <50%), 1 had an intermediate AI (AI 50-60%), and 3 had high AIs (AI >60%). Only one of the 151 IgG positive samples had a low AI, whereas the remaining 150 had high AIs. If only avidity test is taken into consideration, gray region or low avidity test results especially negative for IgM with ELISA and showing a clear chronic pattern will be falsely interpreted in accord with an infection, if the results for these sera are new. IgG avidity test is useful in sera revealing suspected results in IgM ELISA and showing a clear chronological pattern will be falsely interpreted in accord with an infection, if the results for these sera are new. IgG avidity test is useful in sera revealing suspected results in IgM ELISA test. Follow-up of patients with intermediate and low AIs and repeating tests in certain intervals, and examination of the amniotic fluid by PCR will be appropriate. As only IgG avidity test taken into consideration will cause unnecessary abortus and anxiety, use of single confirmation tests in such cases is not right. IgG avidity tests should only be used as confirmation tests in pregnant women.

Introduction
Human cytomegalovirus (HCMV) is among the most important causes of congenital infections. The most important risk in intrauterine infection is the primary or recurrent infection of the mother. In 35-50% of the pregnant women who have primary HCMV infection, intrauterine infections develop in fetus, and 10% of the infected fetuses are born symptomatic; the severity of the clinical scene is related to the amount of virus the baby is infected with, virulence, and the gestational stage. Congenital anomalies in the fetus are more frequent in the first trimester, as organs develop in this period; and death of precursor cells may cause congenital defects (13, 15, 18).

If the mother has rubella infection in the first two weeks of gestation, there is an 80% risk of infection developing in fetus. This risk decreases to 6-10% in the infec-
tions occur until the 14th week. The virus may cause disabilities or embryo deaths in especially the first three months of the gestation. The most prominent effects of Rubella is displayed in the organs in their sensitive development periods. The immunity gained during Rubella infection lasts for a life time. The reason for this is that there is only one antigenic type of the virus present (3, 9, 15).

Diagnosis of HCMV and rubella in pregnant women is performed by detection of IgM and IgG antibodies by various serological tests. The conventional serological diagnosis of a primary infection is based on revealing IgG seroconversion along with IgM positivity. Different IgM responses may also occur against microbial antigens; specific IgM may turn negative in serum in acute infection earlier than expected, or it may be detected for months or years in low titer. Therefore, when specific IgM positivity is detected in a serum sample, it is difficult to decide towards presence of a secondary infection as acute infection, persistent IgM or reactivation/reinfection (4, 9, 17).

In the recent years, as IgG avidity tests got into the practical use, safe discrimination of primary acute infections, reactivation and/or reinfections with a single serum sample became possible. This discrimination holds clinical value especially in pregnant women and immunosuppressed patients (4, 17).

As the standard methods and the time intervals required by these are generally not used properly in the immunoserological diagnosis of various diseases, many difficulties occur in diagnosis and therapy. Generally, titration and increases in the titer of different antibodies in different processes are accepted significant. Detection of this requires a long time. IgG avidity tests, which came on the scene recently with regard to most efficient use of time and early onset to the therapy, are considered in this study, and compared with other classical methods.

**Materials and Methods**

Serums of a total of 879 women in the first trimester of their gestations who referred to Dicle University, Faculty Hospital, Department of Gynaecology and Obstetrics in between the dates of 09 April 2002 – 09 May 2003 were evaluated with the classical method ELISA (Cobas Core II, Roche, USA), in terms of Rubella and HCMV IgG and IgM.

In the IgM positive cases, the same test was repeated one week later with new serum samples. Serums were IgM and IgG were both found positive together, and those positive in terms of IgG only were stored at -20 °C. Avidity tests were performed with (IgG avidity EIA. Well; Radim, Italy) commercial kits.

HCMV IgG avidity test results were evaluated as high avidity where the avidity was higher than 45%, intermediate avidity where avidity read 35-45% (gray region), and low avidity when the avidity was lower than 35%.

Rubella IgG avidity test results were evaluated as high avidity for over 60% avidity, intermediate avidity for those in between 50-60% (gray region), and low avidity for avidities lower than 50%.

**Results and Discussion**

A total of 879 serum samples obtained from women in their first trimester of gestation were evaluated in terms of HCMV and Rubella IgM and IgG with the classical method ELISA. Among the 879 serum samples, 8 was found to be positive for HCMV IgM and IgG (1.1%), 288 for only IgG (42.5%), 13 for Rubella IgM and IgG (1.9%), and 244 for only IgG (36.0%). ELISA test was repeated one week later in patients positive for HCMV and Rubella IgM and IgG with new serum samples, and same results were received. All the serums that were IgM (+) and IgG (+) were studied, whereas those that were IgG (+) only
TABLE 1

The total number of sera with HCMV and IgG avidity results

<table>
<thead>
<tr>
<th>EIA</th>
<th>AP%35&lt;</th>
<th>AP%35-%45</th>
<th>API%45&gt;</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM(+)IgG(+)</td>
<td>8</td>
<td>100.0</td>
<td>4</td>
<td>50.0</td>
<td>2</td>
<td>25.0</td>
<td>2</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>IgM(-)IgG(+)</td>
<td>156</td>
<td>100.0</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>0.6</td>
<td>155</td>
<td>99.4</td>
<td></td>
</tr>
<tr>
<td>Toplam</td>
<td>164</td>
<td>100.0</td>
<td>4</td>
<td>2.4</td>
<td>3</td>
<td>1.8</td>
<td>157</td>
<td>95.7</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2

The total number of sera with Rubella and IgG avidity results

<table>
<thead>
<tr>
<th>EIA</th>
<th>AI%50&lt;</th>
<th>AI%50-%60</th>
<th>AI%60&gt;</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM(+)IgG(+)</td>
<td>13</td>
<td>100.0</td>
<td>9</td>
<td>69.2</td>
<td>1</td>
<td>7.7</td>
<td>3</td>
<td>23.1</td>
<td></td>
</tr>
<tr>
<td>IgM(-)IgG(+)</td>
<td>151</td>
<td>100.0</td>
<td>1</td>
<td>0.7</td>
<td>0</td>
<td>0.0</td>
<td>150</td>
<td>99.3</td>
<td></td>
</tr>
<tr>
<td>Toplam</td>
<td>164</td>
<td>100.0</td>
<td>10</td>
<td>6.1</td>
<td>1</td>
<td>0.6</td>
<td>153</td>
<td>93.3</td>
<td></td>
</tr>
</tbody>
</table>

were studied in accordance with the number of kits left, by IgG avidity test.

As shown in Table 1, 4 samples among the 8 that were positive for HCMV IgM and IgG were found to have low AIs (AI<35%), 2 samples to have intermediate AIs (AI 35-45%), and the other 2 to have high AIs (AI >45%). One of the 156 IgG positive serum samples had an intermediate AI (AI 35-45%), and 155 had high AIs.

In the Rubella IgG avidity tests (Table 2), 9 of the 13 IgM and IgG positive samples had low AIs (AI <50%), 1 had an intermediate AI (AI 50-60%), and 3 had high AIs (AI >60%). Only one of the 151 IgG positive samples had a low AI, whereas the remaining 150 had high AIs.

Whereas the primary infections caused by HCMV and Rubella in the first trimester of gestation cause serious malformations, this risk is minimal or zero in infections caused by reinfeciton and/or reactivation.

In case positivity for HCMV and Rubella IgG together with IgM is detected in a pregnant woman, false qualification of this result as a primary infection leads to unnecessary abortion, whereas false qualification as a secondary infection may cause damage in the fetus. From this point, determination of IgG antibody avidity detected in the mother will be leading the way for primary or reinfection/reactivation. Lutz et al. (14) have detected mean IgG AIs of 12 kidney transplantation patients in whom primary HCMV infection was shown, to be 18% (limit value 30%), and that of 11 patients in whom previously acquired immunity was proved to be 85%; and have reported that this method had a great value in discrimination of primary HCMV infections from prior and/or recurrent infections.

Bodeus et al. (1), in a study evaluating
the IgG avidity performance in the elimination of congenital infection, have examined serum samples from 409 pregnant women positive for IgM and IgG, by ELISA. High avidity eliminating primary infection was observed in 270 women. As 121 women were in the first trimester of gestation, the possibility of primary infection was eliminated in 30% of them.

Grangeot–Keros et al. (6) have reported low HCMV–IgG AIs in all of the 41 HCMV IgM positive pregnant women, whereas high HCMV IgG AIs in all of the 70 pregnant women with history of prior HCMV infection.

Us et al. (18) have observed 41 serum samples among 93 acquired from pregnant women to be HCMV-IgM and IgG positive together, whereas 52 to be HCMV-IgM negative, and HCMV-IgG positive. They found low HCMV-IgG AIs in 34 samples among the group positive for HCMV-IgM and IgG together (82.5%), and high AIs were evident in 7 samples (17.5%). In all the samples belonging to the HCMV-IgM negative and IgG positive group, AIs were detected to be high.

In our study, 4 of the 8 samples (50.0%) positive for HCMV IgM and IgG had low avidities, 2 (25.0%) had intermediate avidities, and 2 (25.0%) had high avidities. Among 156 sera positive for HCMV IgG only, 1 had intermediate avidity (0.6%) whereas the remaining 155 had high avidities. The fact that Lazarotto, Grangeot-Keros and us found high AIs in all of the HCMV-IgM (-) and IgG (+) patients, and that we have detected one case with intermediate AI in the same patient group was attributed to possible delayed avidity maturation.

It is revealed that in all IgM positivities detected together with IgG positivity, avidity tests should be applied. The approximate date of infection may be determined this way. For example, high IgG AI over 60%, together with IgM positivity, will give the idea that the infection is not primary, or at least that it occurred 18-20 weeks before (12, 16).

Hedman and Seppala (8) have found that Rubella IgG avidity was related to the molecular features of the IgG1 subclass, and that the IgG concentration in the serum affected the avidity index. These investigators, in another study, found that IgG avidities were lower than 30% (limit value 40%) in 91.2% of the patients with primary Rubella infection, whereas IgG avidities were higher than 50% in 96.6% of the individuals with reinfection (immune individuals vaccinated or previously known to be seropositive, who have history of infection with virus).

Böttiger and Jensen (2) have investigated IgG avidity levels of 94 IgM positive patients who had typical Rubelliform skin eruptions during Rubella epidemics in Denmark, and have found AIs lower than 30% in 85% of the patients, and AIs lower than 40% in 97% of the patients. In the same study, they have detected the mean AI value as 85% in 137 preimmune women.

Conderelli et al. (5) have reported that use of light denaturing agents such as 6 M urea in the detection of Rubella IgG avidity by ELISA showed the possible date of primary Rubella infection in the two months of sampling in gestation.

Lafarga et al. (10) have examined 178 sera from 157 patients with clinical and/or epidemiologic suspect of Rubella, or with coincidental positive results for Rubella, serologically three different groups consisting of 112 epidemics, 36 pregnant women, and 11 newborns, for 30 months. It was reported that 90.2% of the patients in the epidemics group were not inoculated. It was stated that 92 of the 109 patients with Rubella IgG antibodies revealed AIs lower than 50%, and that the mean rate of IgG Rubella AI was 29.0% in this group. In the pregnant women group, 30 patients were reported to have over 50% AIs, 2 pregnant women to have 37.4% and 20.9% AIs, and
the other 4 pregnant women to have AIs close to the cut-off value, and that the IgG Rubella AI in this group was 71.8%. Comparison of the epidemics group with pregnant women group in terms of IgG Rubella revealed that the difference was statistically significant.

In another study, 84 samples from 15 patients were collected 4-5 months after acute Rubella infections, to reveal the change in time in the avidity of Rubella IgG antibodies following acute Rubella infection, and Rubella specific IgG avidity was studied. AI increased in all patients constantly during the follow-ups, low AI was observed 6 weeks following skin eruption, and high AI was not evident until after 13 weeks from the infection (7).

In our study, 9 (69.2%) of the 13 samples positive for Rubella IgM and IgG had low avidities with AIs lower than 50%, 1 (7.7%) had intermediate avidities with AIs between 50-60%, and 3 (23.1%) had high avidities with AIs over 60%. Only 1 (0.7%) of the 151 samples negative for Rubella IgM, but positive for IgG had an avidity lower than 60%, and the remaining 150 (93.3%) had high avidities with AIs over 60%.

In studies regarding the time required for maturation of Rubella IgG antibody avidity, periods varying between 6-13 weeks were reported. In these studies, IgG avidity index found lower than 40% in patients with primary acute Rubella infection increased to 60-70% in the mentioned periods, and reached to 100% after 38 weeks, increasing in time (6, 18).

When all the studies performed in this subject are examined, Rubella IgG avidity test reveals to be easy and fast, and to eliminate most of the positive results resulting from residual IgM antibodies and false reactivity. It will not be wrong to diagnose a patient having low avidity IgG with IgM positivity, with primary Rubella infection occurred 6 weeks ago, and to diagnose a patient with over 60% avidity detected with IgM positivity, with primary Rubella infection occurred 13 weeks ago.

As results with high AIs in IgG avidity tests eliminates acute infection in the last 3-4 months in cases where the classical ELISA test suggest acute infection for HCMV and Rubella, it gives reliable results in women at 16 weeks of gestation.

If only avidity test is taken into consideration, gray region or low avidity test results especially negative for IgM with ELISA and showing a clear chronological pattern will be falsely interpreted in accord with an infection, if the results for these sera are new. IgG avidity test is useful in sera revealing suspected results in IgM ELISA test.

Follow-up of patients with intermediate and low AIs and repeating tests in certain intervals, and examination of the amniotic fluid by PCR will be appropriate. As only IgG avidity test taken into consideration will cause unnecessary abortus and anxiety, use of single confirmation tests in such cases is not right. IgG avidity tests should only be used as confirmation tests in pregnant women.

REFERENCES