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Reliability of Low-Avidity IgG and of IgA in the Diagnosis of Primary Infection by Rubella Virus With Adaptation of a Commercial Test

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The detection of IgA and low-avidity IgG and antibodies in serum is a potentially useful marker of recent infection by a microorganism. We studied the reliability of IgG avidity and presence of IgA for the diagnosis of recent acute infection by rubella virus. Low-avidity IgG (Avy-EIA test) was determined with a modified commercial test using 8 molar urea (indirect ELISA, DiaSorin, Italy) and IgA was determined with a homemade indirect ELISA test. Twenty-five patients with recent primary infection by rubella virus (group I) and 50 healthy subjects (group II) were studied. In group I low-avidity IgG varied between 100 and 0% (67.3 ± 21.8%); IgA was present in 24 patients (96%). In group II low-avidity IgG varied from 50.4 to 0% (19.8 ± 16.9%). IgA was present in 2 subjects (4%). The sensitivity of the Avi-EIA and the IgA test was 92 and 96%, respectively; specificity was 100 and 96%, respectively. We conclude that both low-avidity IgG and IgA tests are helpful and reliable for the diagnosis of recent primary infection. J. Clin. Lab. Anal. 13:1–4, 1999.

Key Words: rubella virus; avidity; IgG; IgA; serodiagnosis

INTRODUCTION

In most cases of infection by rubella virus, microbiological diagnosis is currently carried out by detecting IgG and IgM antibodies, because direct diagnosis presents technical problems. However, this method is unreliable because false positive and false negative results occur. Immunocompetent adults can be reinfected by the rubella virus as a result of either vaccination failures or mutant strain infection. In these cases patients will not develop an IgM response and elevated IgG titers. Lack of an IgM response in newborns from primarily infected mothers may be caused by immaturity of the immune system, viral antigen blockage by maternal antibodies, infection at a very late stage in pregnancy, or immune tolerance. In the course of infection by the Epstein-Barr virus (1) and Parvovirus B19 (2), an IgM antirubella response may occur, leading to false positive results and a false diagnostic of primary infection by rubella virus. IgM may remain detectable for up to one year (3) after infection. It can also appear in the course of reinfections (4) in immunosuppressed patients, and it may lead to false positives in a low percentage of cases (as many as 3 or 4%) when the rheumatoid factor is present. Finally, it is extremely important to differentiate between primary infection and reactivation, because of the risk to the fetus (5). Most of the IgG produced during primary infection has antigen affinity, which increases with time. Because of this, avidity quantification for specific IgG could represent a new, sensitive,and specific method for the serological diagnosis of recent infection. It also makes the distinction between primary infection and reinfection possible (6–8).

We studied the diagnostic reliability of IgG avidity and amount IgA in recent infection by rubella virus.

MATERIALS AND METHODS

Seventy-five subjects were studied in two groups. Group I was comprised of serum samples from 25 women (age 20± 2 years) showing clinical symptoms of primary infection by rubella virus (fever, adenopathy, and rash) for 1–3 months. Specific IgG and IgM were detected in all cases. Group II was comprised of serum samples from 50 healthy women (age 16 ± 4 years) possessing specific IgG. IgM was not detected in any patient. The women in this group had not been vaccinated within the last six months before serum was collected and none had had a confirmed rubella infection within this period. IgM, IgA, IgG and low-avidity IgG content was assayed.

The IgA assays were carried out using a homemade indirect ELISA: The serum samples were diluted (1:40) with a

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mixture (1/1) of PBS buffer and an anti-IgG antibodies solution (RF-Absorbent®, Behringwerke, Germany, lot 407097). 200 µl of each diluted serum were incubated for one hour at 37°C in a well microtitre plate containing Putnam virus antigen as the solid phase (ETI-Rubek-G®, Sorin Biomedica, Italy, lot 2190710-A) and washed five times with PBS buffer. An antihuman IgA conjugate (antihuman IgA-POD®, Behringwerke, lot 436608) was diluted at 1:50 with PBS buffer and 200 µl of diluted conjugate were then added into each well. The plates were incubated for one hour at 37°C and washed five times with PBS buffer. Then, 100 µl of a mixture 1/10 of Thetramethylene benzidine dihydrochloride (5 g/l) and hydrogen peroxide (0.1 g/l) were added as substrate (supplementary reagents for Enzignost®/TMB, Behringwerke, lot 29363) and the plates were incubated for 30 min at 20°C. The reaction was stopped by the addition of 100 µl of 0.5 N sulfuric acid. The A450 was read with a colorimeter (Diagnostic Pasteur, France). The results were considered positive for A450 > 0.2.

Assays of IgG and IgG avidity were done using a commercial indirect ELISA test (ETI-RUBEK-G®, Sorin Biomedica) and an Avi-EIA test, respectively. Avi-EIA assay is a modified ETI-RUBEK-G® test, which includes a supplementary step to denature the antigen-IgG bond. We carried out this step as follows: The wells were washed after incubation with sera and then 100 µl of 8 M urea were added. The plates were incubated for 5 min at 20°C and washed before addition of the conjugate. The results were calculated using the standard curve described by the manufacturer, and were expressed as IU/ml. The difference between the values obtained from each test (with and without urea) was measured to find the amount of low-avidity IgG, and the results were expressed as a percentage of IgG anti-rubella. The samples were assayed in replicate and the mean value was calculated when the test results differed by 10% or less. To assure the accuracy of the results, the tests were performed once again when the results differed by more than 10%.

IgM was studied using a commercial ELISA capture (ETI-RUBEK-M®, Sorin Biomedica).

We analyzed the reliability of low-avidity IgG levels for the diagnosis of recent acute infection for levels. Previous studies (9,10) reported 55% and 50% as cut-off point values for the diagnosis of recent infection.

RESULTS

Results of Avy-EIA and IgA tests are shown in Figure 1. In group I the percentages of low-avidity IgG varied between 100 and 0% (average 67.3 ± 21.8%); IgA was present in 24 (96%) patients. In group II, the percentages of low-avidity IgG varied from 50.4 to 0% (19.8 ± 16.9%). IgA was present in 2 (4%) subjects who had had previous acute infection by rubella virus within the previous six months; one of them (2%) had a value of 50.4% for low-avidity IgG. The results of Avy-EIA test reliability are shown in Figure 2 as receiver operating characteristic curve. We found the optimum cut-off value to be between 50 and 60% of low-avidity IgG (92% sensitivity and 100% specificity for cut-off value of 55%). For IgA test, both sensitivity and specificity values were 96%.

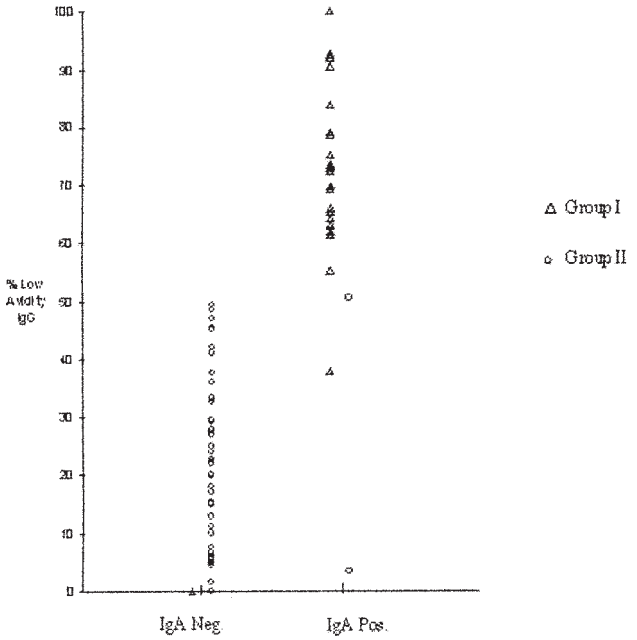

Fig. 1. IgA and low-avidity IgG results for group I (patients primarily infected by rubella virus) and group II (healthy patients).

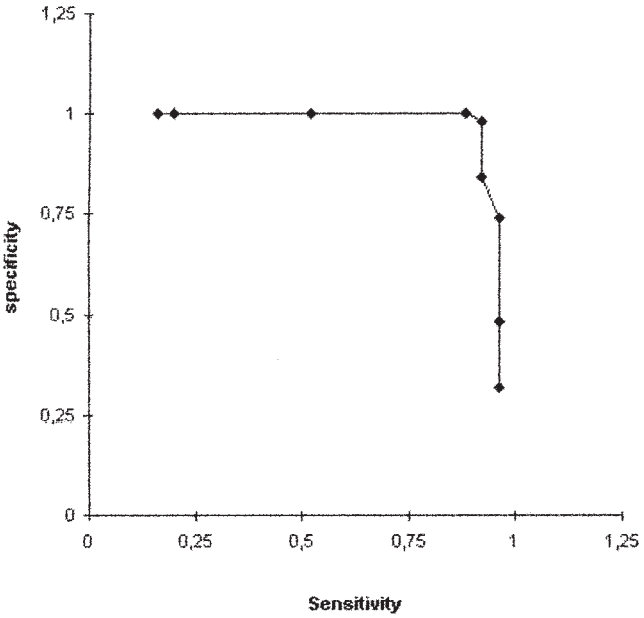

Fig. 2. Receiver operating characteristic curve obtained for low-avidity IgG test. The optimum value was found to be in the range of 50–60%.

DISCUSSION

The IgA test in patients with rubella produces false negative results because of the short life of these antibodies and because IgA serum levels are lower than those of IgM (11). In group I, IgA was not detected in 4% of patients, possibly because it appeared only transiently. In another 4% it was detected when the infection was not in the acute phase. With regard to low-avidity IgG in the control group, 2% of cases had low-avidity antibodies at a fairly low level (50.4%). With the exception of a slightly higher sensitivity (92.8%; 100%) (12,13), these results are comparable to others for the diagnosis of primary infection. However, they indicate less sensitivity in detecting a past infection or reinfection. These relative discrepancies may be explained by the different methods used. In most previous studies, the denaturing substance used for the detection of low-avidity IgG is diethylamine. Diethylamine is more reliable than urea for detecting low-avidity IgG because it denatures the antigen-antibody bond more strongly. Moreover, the assay can be done up to 5–7 months after the onset of the infection. When urea is used, low-avidity antibodies are not usually detected after more than 3 months (14,15). In other studies (16–19), 15–28 days after the onset of primary infection, the percentage of low-avidity IgG detected decreased significantly when urea was used, but not with diethylamine. This might explain why low-avidity IgG was not detected in two cases (8%) whose clinical findings indicated recent primary infections. In our experience, the current results show the time after the onset of primary infection to be extremely important when evaluating the reliability of the test. Both urea and diethylamine methods are reliable when this period is greater than one month.

Nonetheless, these results are better than previously reported findings in which two cases of low-avidity IgG were found among 31 healthy patients and 29 reinfected patients (16–19). In the present study, low-avidity antibodies were detected in only one healthy patient, in which case they were present in very low quantities.

In sera from patients with primary infection by rubella virus, IgM can be detected for longer than low-avidity IgG when the urea method is used (11). With diethylamine, low-avidity IgG also disappears before specific IgM.

The new method should be more effective in detecting cases of reinfection or nonspecific antibodies in the presence of IgM. The test with diethylamine should be more sensitive in detecting primary infection or recent immunization (12,16–20). Some authors have reported that differences in the estimation of IgG avidity are due to epitope density: low-avidity antibodies might better be detected when epitope density is higher and serum concentration is lower. When there is an excess of antibodies, the antigen binding sites are occupied mainly by high-avidity antibodies (16,17). These discrepancies might also be explained by the different conjugate used in each method. By combining the two parameters, when low-avidity IgG and IgA are present, sensitivity is not improved but specificity is. It must be emphasized that most IgA-positive patients in group I had more than 50% low-avidity IgG, and only one patient of the two from group 2 who had an IgA-positive result also had low-avidity IgG. A cut-off point of 55% for the Avi-EIA test is useful in the laboratory.

CONCLUSION

In the case of recent primary infection, most IgGs have low antigen avidity. In the case of past infection, most IgGs have high antigen avidity. Both Avi-EIA and IgA tests are simple and reliable for the diagnosis of recent primary infection. Nevertheless, the IgG avidity and IgA assays should be considered as complementary methods to the determination of IgG and IgM, and the clinical situation of the patient must be taken into account to avoid false negative and false positive results. In those cases in which the avidity result does not distinguish between recent and past infection, demonstrating the disappearance of low avidity-IgG might solve the dilemma.

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