Detection of Low-Avidity Immunoglobulin G in Oral Fluid Samples: New Approach for Rubella Diagnosis and Surveillance

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Low-avidity rubella immunoglobulin G (IgG) was detected in oral fluid samples from 30 of 32 rubella IgM-positive patients (sensitivity, 94%) and from 4 of 34 IgM-negative patients (specificity, 88%). Measuring IgG avidity in oral fluid samples could improve the reliability of rubella surveillance when the incidence of the disease and the positive predictive value of IgM tests are low.

Testing of oral fluid samples instead of serum samples offers many advantages for surveillance (5). This fact led in 1994 to its implementation for the laboratory confirmation of rubella cases reported by general practitioners in the United Kingdom (6). A case is confirmed if rubella immunoglobulin M (IgM) is detected in oral fluid. At present, however, the true incidence of rubella infection is low (4); therefore, the positive predictive value (PPV) of specific IgM tests for confirming a recent infection is also low (3). The measurement of IgG avidity is an alternative to an IgM assay for confirming recent rubella and for distinguishing primary from secondary infections (8). We now report that measuring rubella IgG avidity is feasible with oral fluid samples.

This study was performed with 66 oral fluid samples collected for the laboratory confirmation of reported rubella cases as described previously (6). The rubella IgM radioimmunossay was positive (test sample count/negative control count [T/N ratio, >3]) for 32 oral fluid samples, indicating recent infection; 34 oral fluid samples were found to be IgM negative (T/N ratio, <3) and IgG positive by the radioimmunosassay, indicating past infection or vaccination. The samples were collected from patients presenting with rash and fever during a rubella epidemic in the United Kingdom in 1996 (7) and were stored at −20°C; in this epidemiological context, the PPV was high. Of the 32 IgM-positive patients, 30 had not received a rubella-containing vaccine, and there was no available information on vaccination status for the remaining 2 patients. Of the 34 rubella IgM-negative patients, 23 had a history of vaccination on vaccination status for the remaining 2 patients. Of 30 IgM-positive samples with low-avidity IgG, excluding recent infection compared to oral fluid IgM detection. For an IgG avidity index of <60%, 2 and 30 samples were found to be IgM positive and IgM negative, respectively; for an IgG avidity index of >60%, 2 and 30 samples were found to be IgM positive and IgM negative, respectively. Regression analysis of the avidity index against months before the samples were collected.

The avidity of rubella IgG was measured by a modification of an IgG capture enzyme-linked immunosorbent assay (9). After the binding of oral fluid IgG to solid phase anti-human IgG, followed by the addition of rubella antigen, 6 M urea (to elute low-avidity IgG) was added to one of duplicate test wells and phosphate-buffered saline (PBS) was added to the other; the test plate was shaken for 10 min at 37°C on a microtiter plate shaker. After two washes with PBS containing 0.5% Tween 20, the assay was developed as previously described (9). The optical density at a wavelength of 450 nm (OD450) was measured by using a reference wavelength of 620 nm (OD620) for the urea-treated test well (OD450/620) and compared to that for the PBS-treated test well (OD450/620 PBS) by using the following formula to calculate an avidity index: (OD450/620 UREA/OD450/620 PBS) × 100. When OD450/620 UREA exceeded OD450/620 PBS (i.e., there was no reduction of OD450/620 UREA), the avidity index was taken to be 100%.

The IgG avidity index in oral fluid samples from patients confirmed by the IgM assay to have had recent rubella (mean, 33.7%; range, 17.2 to 71.9%) was significantly lower than that in oral fluid samples from patients for whom recent infection was not confirmed (mean, 75.3%; range, 28.5 to 100%; P < 0.0001 [t test]). The distribution of IgG avidity reactions in IgM-positive and IgM-negative oral fluid samples suggested that an avidity index of 60% distinguished recent from past infection (Fig. 1). With this cutoff value, the oral fluid rubella IgG avidity assay had high sensitivity (94%) and specificity (88%) for confirming recent infection compared to oral fluid IgM detection. For an IgG avidity index of <60%, 30 and 4 samples were found to be IgM positive and IgM negative, respectively; for an IgG avidity index of >60%, 2 and 30 samples were found to be IgM positive and IgM negative, respectively. Regression analysis of the avidity index against days after onset of illness in patients with low-avidity IgG showed some evidence (P, 0.046; correlation coefficient, 0.34) that the avidity index increased with time after onset. The regression equation gave an estimate for the mean avidity index of 29% at 10 days after onset; this value increased to 40% at 50 days after onset.

The 30 IgM-positive samples with low-avidity IgG were collected between 4 and 48 (mean, 18.9) days after the onset of illness. Two samples, collected 3 and 26 days after onset, had high-avidity IgG (avidity indices, 65.7 and 71.9%, respectively) but were IgM positive (T/N ratios, 25.1 and 4.3, respectively). Thirty IgM-negative samples had high-avidity IgG, excluding recent infection. Discordant results were obtained for four samples, collected 19, 32, 44, and 46 days after onset; these had low-avidity IgG (avidity indices, 59.9, 28.5, 45.9, and 30.3%, respectively).

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respectively) but were IgM negative (T/N ratios, 1.1, 2.4, 2.3, and 1.9, respectively).

The measurement of low-avidity virus-specific IgG has become the method of choice for the serological diagnosis of primary infection with a number of viruses, including hantavirus (2) and human herpesvirus 6 (10). The method also has an important role in distinguishing primary from secondary infections, for example, in the diagnosis of suspected rubella infection in pregnancy (1). We have now demonstrated that the pattern of rubella IgG avidity in oral fluid samples parallels the pattern observed in serum samples. This is the first description of the measurement of low-avidity virus-specific IgG in oral fluid samples and also the first use of a capture assay format to measure IgG avidity.

The detection of specific IgM and low IgG avidity in oral fluid samples offers a confirmatory strategy for rubella diagnosis with a single noninvasive sample. Other recent studies have shown that, in addition to its use for antibody assays, oral fluid is a suitable sample for the PCR detection of rubella virus (L. Jin, A. Vyse, and D. W. G. Brown, Letter, Bull. W. H. O. 80:76–77, 2002). These new approaches expand the potential of oral fluid to serve as a sample for viral diagnosis, although the application of PCR testing for rubella is limited to the acute phase of the disease (<14 days after onset) and good specimen preservation is required for optimal performance (Jin et al., letter). In the study described here, low-avidity rubella IgG could be detected for up to 48 days after the onset of illness. The availability of supplementary tests for confirming the specificity of positive rubella IgM results obtained with oral fluid samples is especially useful when the true incidence of the disease is low, as is currently the case for rubella in the United Kingdom. By increasing the reliability of laboratory diagnosis of reported cases, oral fluid rubella IgG avidity testing could contribute to the control of rubella.

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