

Diagnosis of rubella infection by detecting specific immunoglobulin M antibodies in saliva samples: a clinic-based study in Niterói, RJ, Brazil

Diagnóstico laboratorial da rubéola através da detecção de imunoglobulina M específica em amostras de saliva

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Abstract *This study was designed to investigate whether saliva could be a feasible alternative to serum for the diagnosis of recent rubella infection in a clinic setting. Forty-five paired blood and saliva samples collected 1 to 29 days after onset of illness were tested for specific immunoglobulin (Ig) M by antibody-capture radioimmunoassay (MACRIA). Rubella IgM was detected in all serum samples and in 38 (84.4%) saliva specimens. Forty-six serum and saliva samples from other patients with rash diseases were tested by MACRIA for control purposes and two saliva specimens were reactive. The saliva test had specificity of 96%. These results indicate that salivary IgM detection may be a convenient non-invasive alternative to serum for investigation of recent rubella cases, especially for disease surveillance and control programmes.*

Key-words: Rubella. Saliva. Diagnosis. IgM. Antibody-capture radioimmunoassay.

Resumo *Este estudo foi realizado para avaliar a validade da utilização da saliva no diagnóstico laboratorial da rubéola. Quarenta e cinco amostras pareadas de sangue e de saliva, coletadas de 1 a 29 dias após o início da doença, foram testadas para detecção de imunoglobulina (Ig) M específica por radioimunoensaio com captura (MACRIA). Anticorpos IgM específicos contra rubéola foram detectados em todas as amostras sanguíneas e em 38 (84,4%) das amostras de saliva. A especificidade do teste na saliva foi de 96%. Estes resultados indicam que a utilização da saliva pode ser uma alternativa válida para obtenção de espécimens clínicos na investigação de casos recentes de rubéola, especialmente nas atividades de vigilância epidemiológica e controle da virose.*

Palavras-chaves: Rubéola. Saliva. Diagnóstico. IgM. Radioimunoensaio.

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The infection caused by rubella virus is usually mild or asymptomatic in adults and children. However, rubella infection in pregnant women, especially during the first trimester, can result in miscarriage, stillbirth, and congenital defects¹⁹. Live attenuated rubella vaccines were licensed in USA in 1969 and have been used widely in many industrialised countries since that time^{7 20}. Since 1987, some states in Brazil have added rubella vaccines to their immunisation programmes, using a variety of different schedules. A national programme to control the disease and congenital rubella syndrome (CRS) was introduced in 1997 by implementation of rubella vaccination and surveillance of CRS and acquired rubella cases⁶.

The features of rubella infection overlap considerably with other viral infections that present erythematous rashes, and it is sometimes difficult to make a diagnosis on clinical grounds alone. Laboratory diagnosis of recent or past rubella

is particularly important for pregnant women and for the design and monitoring of vaccination programmes^{3 21}.

However, the requirement to take blood samples is not always practical, and limits its widespread use, particularly for those populations outside the clinical environment and for children^{11 13 21}. The use of saliva as a non-invasive alternative to serum for detecting virus specific antibodies was first described by Parry et al¹⁵. Subsequently, specific IgM in saliva was detected successfully in patients with recent hepatitis A or B¹⁶, measles, mumps, and rubella¹⁷, human parvovirus B19⁴, and dengue fever¹⁴.

The objective of this study was to investigate whether saliva could be a feasible alternative to serum for the diagnosis of recent rubella infection under conditions in routine health care centres in Brazil.

MATERIAL AND METHODS

From January 1994 to January 1999 a study of the etiology of exanthematic diseases was conducted at the Department of Infectious Diseases of the Hospital Universitário Antonio Pedro, Niterói, RJ, and two other large Health Care Units from that Municipality. All patients seen at the selected study sites with an acute maculopapular rash were asked to participate and to consent to the collection of the specimens. A total of 473 cases were seen and 19.6% (94 cases) of them was confirmed by rubella specific IgM testing using a commercial ELISA (Rubenostika IgM, Organon). Paired blood and saliva samples collected simultaneously were available from 45 (47.9%) of the 94 rubella IgM positive cases. To allow specificity assessment, paired blood and saliva samples from 49 patients with other recent rash diseases (n measles, n parvovirus B19, n dengue) were tested.

A commercial device (OraSure, Epitope, Bearverton, OR, USA) was used to collect saliva specimen. According to the manufacturer's instructions, a small pad of absorbent material was placed into the patient's mouth between the lower cheek and gum and stroked back and forth several times until moistened. Then the pad was held in place for 2 min with the mouth closed. After that the pad was removed and inserted in the bottom of a vial containing preservative. The samples were kept refrigerated before being transported to the laboratory. Saliva was extracted from the absorbent pads by centrifugation (2500rpm for 15min) and stored at -20°C before testing.

Rubella IgM antibody assay. An antibody-capture radioimmunoassay (MACRIA) was used to detect rubella specific IgM in saliva and serum samples as previously described¹⁷. Briefly, polystyrene beads coated with rabbit antibody to human IgM (DAKO, Denmark) were incubated with either undiluted saliva samples or serum samples at a final dilution of 1 in 100. After washing with phosphate buffered saline with 0.05% Tween-20 (PBST) using the Abbott Qwikwash System (Abbott Diagnostics, Chicago, IL, USA), rubella haemagglutinin antigen [Judith strain, HA titre 1:256, Batch n° 2168/1 (Laboratory of Microbiological Reagents, CPHL, Colindale, UK)] was incubated with the beads overnight at 4°C. The beads were then washed with PBST, and monoclonal antibody to rubella haemagglutinin (Laboratory of Microbiological Reagents) was added and incubated for 2h at 37°C. Finally the beads were washed in PBST and incubated for 2h at 37°C with ¹²⁵I-labelled sheep anti-mouse IgG (Code IM 131, Amersham International, UK). Beads were washed and the bound radioactivity was measured in a gamma counter. Specimens were considered positive for IgM if they gave a test: negative (T:N) ratio • 3.0. T:N values < 3.0 were regarded as negative for this study¹⁷.

Total IgG assay. Saliva total IgG content was determined to verify the adequacy of the specimen, by using an enzyme immunoassay (EIA) as described before².

Data analysis. The performance of rubella MACRIA on saliva samples was assessed by calculating (a) the proportion of individuals with rubella IgM in saliva among those with rubella

IgM in serum (sensitivity) and (b) the proportion of individuals without detectable rubella IgM in saliva among those rubella IgM negative in serum (specificity).

RESULTS

Forty-five (47.9%) of the 94 rubella cases seen in the participating institutions were enrolled in this study. Paired blood and saliva samples were taken from the 45 cases from 1 to 29 days after onset of illness. No patient or patient's parents refused permission for sampling to be performed. The ages of the patients ranged from 1 to 46 years, with 56.6% (25 cases) below 15 years old. The most common symptoms found to be associated with rubella infection were rash

(100%), pyrexia (68.9%), post-auricular and/or occipital lymphadenopathy (60%), cough and/or coryza (57.8%) and arthropathy (37.8%). Of the 45 rubella cases, only 1 had a history of MMR (measles, mumps, and rubella) vaccine in the past.

Overall, rubella-specific IgM was detected in 38/45 (84.4%) saliva samples of patients with rubella-specific IgM in serum. Table 1 shows the detection of virus-specific IgM in saliva according to the day of onset of illness.

Table 1 - Day of onset of illness and frequency of rubella-specific IgM in saliva specimens from serologically confirmed cases of rubella and other rash diseases negative to rubella-specific IgM.

Day of onset of illness	Rubella-specific IgM in serum		Other rash diseases	
	rubella-specific IgM	total	rubella-specific IgM	total
	in saliva	seropositive	in saliva	seronegative
1-5	14	15	-	21
6-10	16	18	2	17
11-29	8	12	-	8
Total	38 (84.4%)*	45 (100.0%)	2 (4.3%)*	46 (100.0%)

* Sensitivity; # False-positive rate.

For control purposes, 49 paired blood and saliva samples collected from patients with diseases with rash not related to rubella infection (serum rubella IgM negative by the commercial ELISA mentioned above) were also tested. The specimens were collected within 30 days after the onset of disease. The age of the patients ranged from 15 days to 36 years. Forty six sera were negative by rubella MACRIA but 3 sera gave repeated reactivity (T:N 3.7, 3.2, 7.3), while corresponding saliva specimens were unreactive in 2 cases and weakly reactive (T:N 3.3) in 1 case. As we were unable to evaluate the discordant results either by using a third rubella IgM test or to demonstrate rising titres of rubella antibody in

paired serum samples or to demonstrate low avidity rubella IgG, the three cases were regarded as inconclusive and excluded from the analysis. Of the remaining 46 paired blood and saliva samples taken for control purposes, 2 saliva specimens showed some rubella-specific IgM reactivity (T:N 4.8, 5.0). The predictive value of a positive test for saliva rubella IgM in this study was 95% and the predictive value of a negative test was 86.3%.

The concentration of total IgG saliva specimens was assessed in 6 of the 7 false negative salivary rubella specific IgM samples. One out of six samples contained low levels of total IgG (4.0mg/l). For the other 5 samples, the total IgG concentration ranged from 9.1 to > 58mg/l.

DISCUSSION

Some studies about detection of rubella class-specific antibodies from saliva specimens are described in the literature^{1 5 13 17 18 21}. Perry et al¹⁷, using antibody-capture radioimmunoassay, showed that virus specific IgM was detected in 100% of rubella saliva samples collected between

1 and 5 weeks after onset of disease. Ramsay et al¹⁸ in a community-based study of notified cases of rubella in England and Wales found that the sensitivity of saliva rubella IgM testing was 81%. However, the sensitivity rose to 90% when results from specimens collected outside the recommended

period (1-6 weeks after onset) and specimens taking more than 1 week to reach the laboratory were excluded. Our results are similar to those reported by Ramsay et al¹⁸. Rubella specific IgM was detected in 84.4% of saliva specimens, demonstrating high level of concordance between blood and saliva IgM results. The saliva test had a specificity of 96%.

Inadequate saliva collection could have contributed to a missed salivary diagnosis. However, in 5 of the 7 false negative cases the total IgG concentration indicated adequate samples. Notwithstanding that Perry et al¹⁷ have stated that salivary concentration of total IgG is an adequate criterion for assessing the quality of the specimens, other studies have suggested that inadequate saliva samples might not explain false negative IgM results¹⁴.

As stated above, timing of salivary specimen collection is another important factor in determining whether viral specific IgM will be detected^{3 18}. However, this factor does not seem to have contributed to miss salivary diagnosis in the present study: only 1 out of the 7 false negative IgM saliva samples was obtained outside the recommended period¹⁷.

The results presented in this study indicate that saliva may be a viable alternative to serum for community rubella surveys and surveillance in Brazil. Besides the introduction of a national programme to control the disease and CRS in 1997, accurate surveillance of acquired rubella and CRS has been a critical component of the Brazilian Vaccine Programme⁶. The mildness of the majority of rubella cases makes parents and medical practitioners reluctant to take blood for diagnosis. Moreover, in developing countries rubella outbreaks can occur with no clinical recognition, even in a community in which health is being monitored⁹. The use of non-invasive specimens for diagnosis offers several advantages over blood such as: acceptability to patients, applicability to children¹⁰, reuse of disposable equipment is avoided and the occupational risk from needlestick injuries is eliminated⁸.

The present work and other recent studies^{1 12 18 21} indicate that saliva is a viable alternative to serum for monitoring the impact of vaccination programmes in the future. These results highlight the need of development of simple assays such as enzyme-linked immunosorbent assay for salivary rubella IgM for use in public health laboratories worldwide.

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