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Serologically proven acute rubella infection in patients with clinical diagnosis of dengue

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SUMMARY

Patients with a clinical diagnosis of dengue but negative by serological testing were studied for rubella infection. Paired sera were obtained from 69 patients during an outbreak in Yucatán, México. The presence of specific anti-viral IgM in the acute sera was considered as diagnostic for rubella infection. The immunoglobulin was determined by measuring the difference in the inhibition of hemagglutination between the non-reduced and the reduced fractionated sera. Immunoglobulins were separated by sucrose density centrifugation. Acute rubella infection was found in 7 (10·1%) of the patients. These results demonstrate active rubella infection in patients clinically diagnosed as dengue.

INTRODUCTION

Rubella and rubella-like infections cannot be differentiated clinically: particularly when cases occur simultaneously with a rubella-like infection outbreak [1]. A differential diagnosis is needed when pregnancies and implementation of vaccination programmes are involved [2].

In México, dengue and rubella infections can occur simultaneously. Dengue appears to be epidemic with seasonal peaks in the majority of the states of México. Since 1978 the reported incidence has increased from 0·05 to 76 cases per 100000 inhabitants. Rubella is an endemic infection all over the country with reported incidence between 3·9 to 69 cases per 100000 inhabitants. No epidemic outbreaks of rubella were reported from 1969 to 1988 [3–5]. Furthermore, México does not include rubella in its vaccination programme [6].

Rubella infection can only be confirmed by specific viral tests: isolation of the virus, seroconversion or the identification of IgM anti-rubella antibodies [2]. The detection of specific anti-rubella IgM is considered by the World Health Organization as the only valid criteria to distinguish between primary and secondary infection [7]. The presence of anti-viral IgM in acute sera can be

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demonstrated using numerous methods; though fractionation of immunoglobulin by density gradients, combined with reduction with mercaptoethanol and titration by inhibition of hemagglutination is highly specific and sensitive [8].

To detect cases of acute rubella during a dengue infection outbreak, we decided to search for specific IgM anti-rubella in patients who presented with a diagnosis of dengue but were negative in serological tests. Dengue virus type 4 and 1 were isolated from some patients who were positive in clinical and serological tests [9].

MATERIALS AND METHODS

Patients

During the 1984 dengue outbreak in Yucatán 69 patients were investigated. They were a sector of 200 cases diagnosed of having dengue on clinical grounds: high fever, headache, bone pain, anorexia, nausea and generalized macular or mottled rash [10]. By serological tests using inhibition of hemagglutination [11], 131 sera were positive and 69 showed no acute dengue infection. Positive dengue was considered when there was at least a fourfold increase in titre between the acute and convalescent sera [10].

Sera

Acute sera was collected during the first 2 weeks of the clinical observations and convalescent sera from the second to the fifth week. The samples were obtained from patients treated at the Centro de Investigaciones Tropicales, Mérida Yucatán. The sera were stored at −20 °C until tested.

Hemagglutination inhibition assay (HIA)

This test was performed according to the standard procedure of the Centers for Disease Control [12]. Rubella antigen was obtained from Therein virus propagated in the laboratory in Vero cells [13]. Anti-rubella antibody content was expressed in International Units per millilitre of IgG specific for rubella hemagglutinin (IU/ml). Reference sera were obtained from the Laboratoire de Santé Publique du Québec, Canada. The minimum antiviral antibody concentration considered protective was 15·6 IU/ml [14].

Serum fractionation by sucrose gradient

The immunoglobulins were separated by ultracentrifugation in sucrose gradients, according to the standard technique of the Centers for Disease Control [12]. Every fraction was titrated, treated with 2-mercaptoethanol (ME) according by Vesikari's method [15] and titrated again. At the same-time the refractive index was measured for each fraction.

RESULTS

Sera content of anti-rubella immunoglobulin

The acute and convalescent sera were titrated by HIA and the anti-rubella immunoglobulin content expressed in IU/ml. Of the paired sera, 54 (78·2%) were

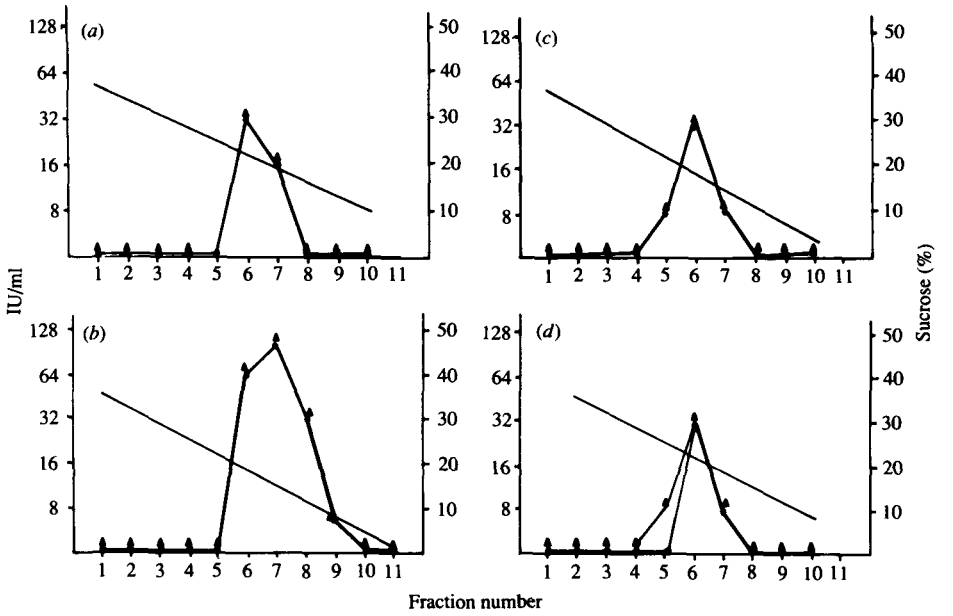

Fig 1. HIA titre of the sucrose density fractionated immunoglobulins. (●), Non-reduced; (▲), reduced fractions. (*a*), Sera with only IgG; (*b*), sera 18; (*c*), sera 50; (*d*), sera 60. The sucrose is shown (as a percentage) in the continuous line.

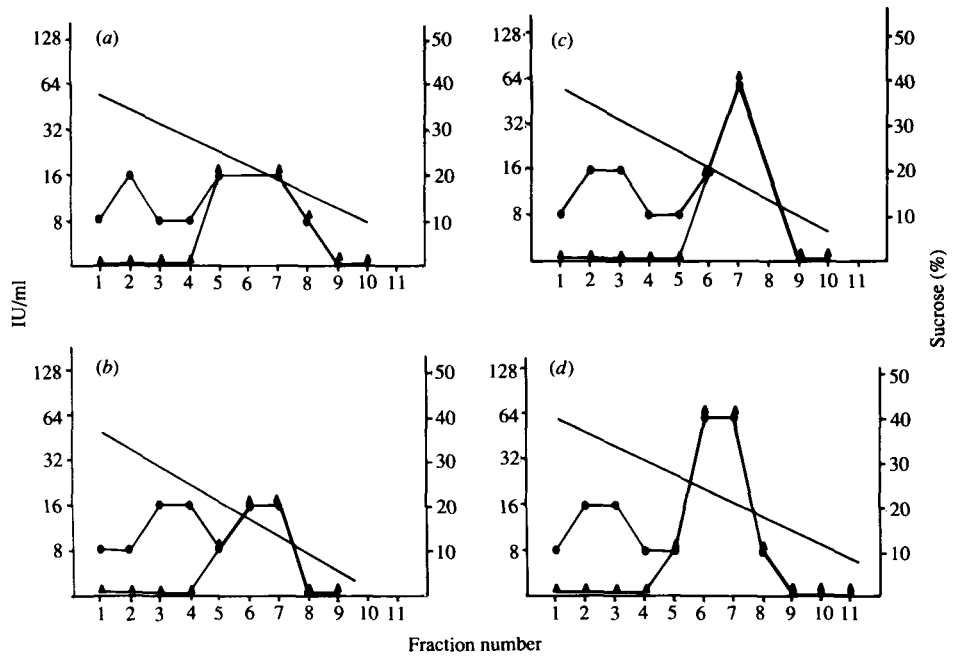

Fig 2. Same as Fig 1. (*a*), sera 5; (*b*), sera 6; (*c*), sera 10; (*d*), sera 52.

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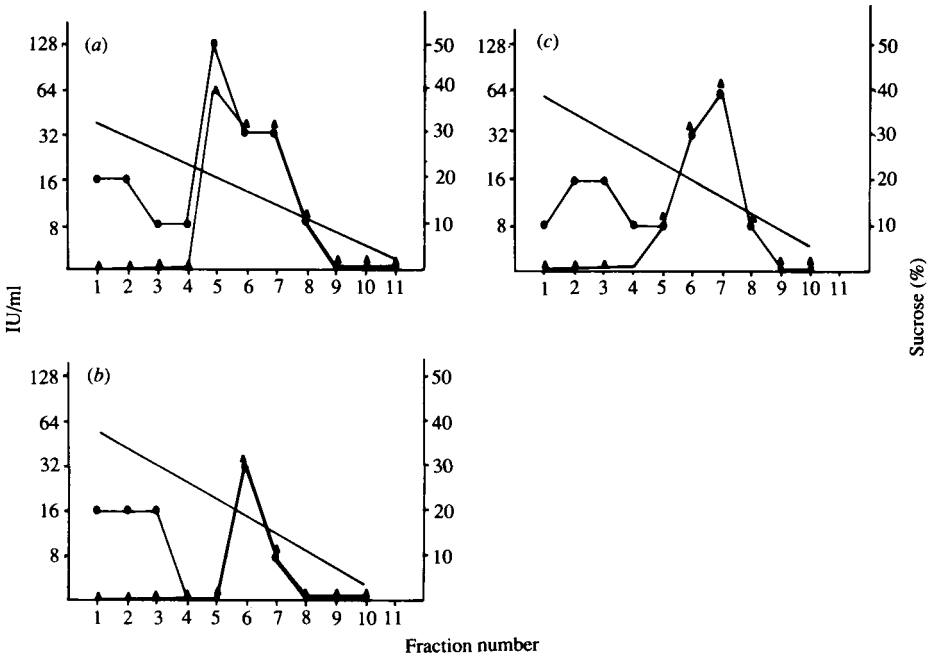

Fig 3. Same as Fig 1. (*a*), sera 54; (*b*), sera 62; (*c*), sera 66.

positive. A wide range of antibody concentration in the sera was found: from negative to 1000 IU/ml. A difference in HIA titre between the acute and convalescent sera was observed in 4 (5·8%) of the cases. Such rises were found only with acute sera which had titres equal or higher than 125 IU/ml.

Of the patients studied, 15 (21·7%) could be considered rubella susceptible, as the anti-rubella antibody content was below the lowest protective level of 15·6 IU/ml. In 11 (15·9%) of the subjects, the concentration of anti-rubella immunoglobulin was below the sensitivity limit of the technique.

Detection of specific rubella IgM

Sera were selected for determining the presence of specific IgM anti-rubella on the HIA titre. Acute sera with a titre equal to or greater than 125 IU/ml and samples which had an increase in titre during convalescence were included in the study.

Of the 69 acute sera, 10 had HIA titres indicating the presence of specific anti-rubella IgM. The presence of specific anti-rubella IgM was confirmed by fractionation, titration, reduction and re-titration. The criteria for establishing the IgM presence were based on the comparison of the non-reduced and reduced fractions. A decrease in the titre in the reduced fractions containing IgM was considered to be positive. Under these conditions the IgM antiviral antibody was found in the first five fractions and the anti-hemagglutinin IgG in fractions 5 to 9 (Figs. 1, 2 and 3).

No significant difference between the non-reduced and reduced samples (Fig. 1*b–d*) was observed in sera 18, 50 and 60 suggesting the absence of IgM. However,

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in sera 5, 6, 10, 52, 54, 62 and 66 the ME treatment caused a significant decrease in the HIA titre in the reduced fractions, indicating the presence of IgM. The results obtained are shown in Fig. 2(*a–d*) and Fig. 3(*a–c*).

Furthermore, no drop in titre between the non-reduced and reduced samples was seen in the IgG-containing fractions (6 to 9). In these samples the ME treatment had no effect in the HIA titre.

DISCUSSION

The possibility of confusing the diagnosis of rubella and dengue on clinical grounds is well documented [16]. However, the presence of specific IgM anti-rubella in patients diagnosis as having dengue has not been reported. Moreover, a study during a dengue outbreak where the virus was isolated from the positive dengue cases has not been done.

The results demonstrate that clinically rubella and dengue infections can not be distinguished. Furthermore, they highlight the difficulty of a differential diagnosis in clinical observations between rubella and rubella-like infections. Rubella is misdiagnosed frequently and may be commoner than has been assumed. In the population studied, one of every 28·5 patients diagnosed as having dengue had active rubella infection.

The etiological agent responsible for the remaining non-rubella cases was not investigated further; perhaps enteroviruses are involved in a proportion of the patients [1].

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