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Enzyme Linked Immunosorbent Assay of Rubella Antibody

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Serum rubella IgG and IgM antibodies were determined in normal healthy women and in rubella patients using rubella Enzyme Linked Immunosorbent Assay (ELISA) kits.

Fifty four per cent (67/125) of healthy women had IgG rubella antibodies.

Positive correlation was found between rubella ELISA IgG antibody and rubella hemagglutination inhibition (HI) test.

Elevation of rubella IgM antibody was demonstrated during acute phase of the rubella infection.

It was concluded that the ELISA of rubella antibody can be useful in surveying the immune status of a population before performing vaccination and in diagnosing rubella during acute phase.

(Key Words: Rubella vaccine, Rubella IgM antibody, Rubella infection)

INTRODUCTION

Rubella infection in early pregnancy results in intrauterine infection of the fetus, and can result in the birth of a baby with congenital rubella syndrome. Prevention of congenital rubella syndrome may be done in two ways: 1) vaccination of women before child-bearing age. 2) correct diagnosis of rubella infection and termination of pregnancy if rubella had been recently contacted.

ELISA is one of the simplest and the most sensitive methods for detecting antibody to rubella (1-3, 5). The purpose of this study was to evaluate commercially available ELISA kits for measuring IgG and IgM antibodies to rubella as a means of surveying the immune status before rubella vaccination and of diagnosing recent rubella infection.

MATERIALS AND METHODS

Sera: Sera from 125 normal healthy women, who were attending a women's college in Kanagawa and from three rubella patients, who visited out patient clinic of Tokai University Hospital during 1982 were used. Those sera were stored at $-20°C$ until assayed.

ELISA of IgG and IgM antibodies: Commercially available ELISA Kits (Rubelisa and Rubelisa M by M.A. Bioproducts, Walkersville, Maryland, U.S.A. :available in Japan through Asahi Medical Co., Shinagawa, Tokyo) were used. The assay kits contained microelisaplates composed of 96 wells. Rubella antigen was fixed to the wells in rows A, C, E, and G. Control antigen was fixed to the rest of the wells. Patients' serum specimen, with (IgM

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assay) or without (IgG assay) pretreatment with Staphylococcus aureus Cowan 1, were added to the wells fixed with rubella antigen or with control antigen. The tray was placed at room temperature for 45 minutes. After the 45 minutes incubation, liquid was removed from the wells and the tray was washed three times with phosphate buffered saline containing Tween (PBS-Tween). Alkaline phosphatase conjugated (rabbit) anti-human IgM or IgG was then added to each well. After 45 minutes incubation, the liquid was tipped out and the tray washed as described above. Para-nitrophenyl phosphate enzyme substrate solution was added to each well. After 45 minutes, 1 N NaOH was added to each well to stop the enzyme-substrate reaction. Absorbance at 405 nm was measured by a microplate spectrophotometer (MTP-12, Corona Electric Co., Katsuta city, Ibaragi, Japan). Absorbance values of each serum's control antigen reaction were subtracted from the corresponding absorbance values of the serum's rubella antigen reaction.

HI assay: Commercially available HI Kits (Takeda Chemical Industries, Osaka) were used.

RESULTS

Figure 1 shows the optical densities of rubella ELISA in 125 healthy women. The cut off value was set at 0.17 in this kit, hence 67 out of 125 persons were found to be positive for rubella IgG antibody. Optical densities of most of the negative samples were between -0.05 and 0.05. Optical densities of most of the positive samples were more than 0.20. Ten samples were considered to be borderline since the optical densities of those samples were between 0.10 and 0.20, which was very close to the cut off value. Figure 2 shows the results of HI test and ELISA in 35 sera. All the samples with HI titers of more than 1:128 were positive by ELISA IgG. All the samples with HI titers of 1:8 or less were negative by ELISA IgG test. The samples with HI titers between 1:16 and 1:64 were judged either negative or positive by ELISA IgG test. Strong correlation (correlation coefficient: 0.92) was found between ELISA optical densities and HI values.

Figure 3 shows the rubella IgM antibodies measured by ELISA in three rubella patients. Substantial increases of rubella IgM antibodies were present during the acute phase of rubella infection.

DISCUSSION

Since 1977, female junior high school students, susceptible to rubella infection are required to receive rubella vaccination in Japan. To survey the immune status the H I test has been the method of choice because this method is sensitive enough to select out those who do not need vaccination. Our study revealed that the ELISA method can also be used as a screening test before vaccination.

Our preliminary study with rubella ELISA IgM showed that measurements of ELISA IgM values of a paired serum specimen of patients with acute rubella infection are useful for diagnosis of acute rubella infection. However, a positive ELISA IgM value of a single serum specimen does not necesarily mean a recent rubella infection, because ELISA IgM antibodies continue to be present in the sera of some patients with rubella infection

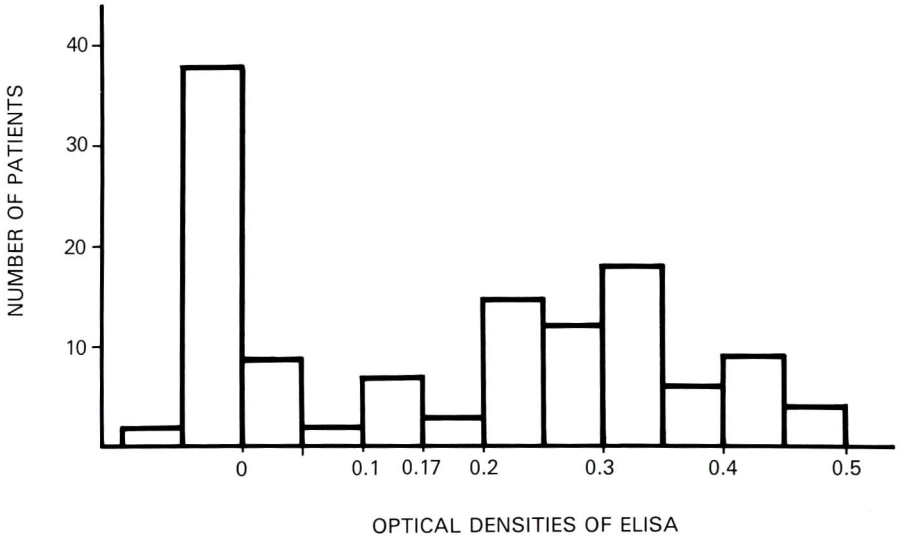

Fig. 1 Histogram of optical densities of rubella ELISA IgG in 125 normal healthy adult women.

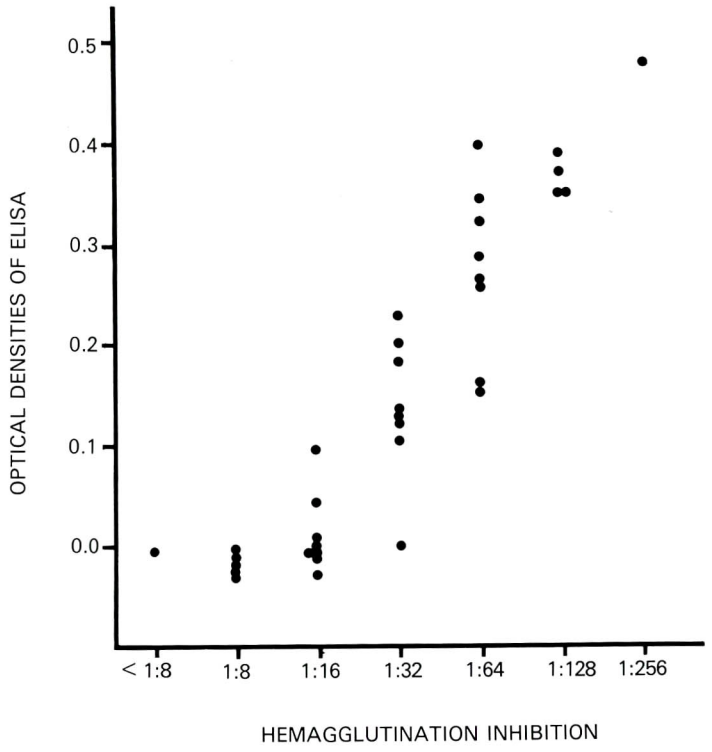

Fig. 2 Optical densities of rubella ELISA IgG and HI tests in 35 serum samples.

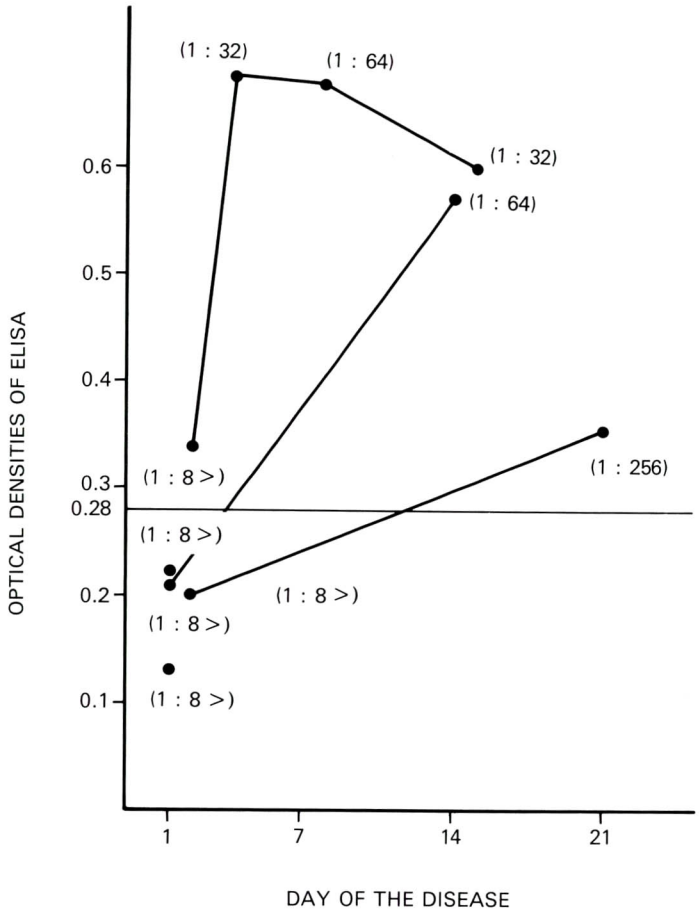

Fig. 3 Optical densities of rubella ELISA IgM in patients with acute rubella infection. Results of HI tests are shown in parentheses.

for three months or more (4). In ELISA IgM, rheumatoid factor (IgM) interferes with the assay by binding with specific IgG antibodies which have already reacted with antigen and thus giving rise to a pseudopositive reaction. In Rubelisa M assay procedure, pretreatment with Staphylococcus aureus Cowan I is present. This process removes IgG (except IgG_3). The pseudopositive reaction by rheumatoid factor is prevented by removing specific IgG. Effects of other nonspecific reactants in the ELISA IgM assay have not been fully evaluated. Further study may be needed before concluding that this method is specific and sensitive enough to diagnose recent mumps virus infection.

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