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SHORT COMMUNICATION

CASE REPORT OF CONGENITAL RUBELLA IN OSAKA PREFECTURE

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Widespread rubella epidemics were observed in the United States (CDC, 1964) and Okinawa (Ueda et al., 1967) resulting in a large number of infants with congenital rubella syndrome. However, there are few reports of even sporadic cases of congenital rubella in Japan. If the incidence of congenital rubella is really low in Japan elsewhere than in Okinawa, an interesting problem is the reason for this. In studies on this problem, we have been trying to detect real cases of congenital rubella in Osaka prefecture virologically. This communication describes clinical and virological findings on some sporadic cases of congenital rubella confirmed serologically for the first time in Osaka.

The patients were born between January 1968 and February 1970 with congenital anomalies following maternal rubelliform or subclinical infection in the early stage of pregnancy, and they were observed at the Children's Medical Center of Osaka City, Kihoku branch of Wakayama Medical College and Osaka Municipal Momoyama Hospital. Throat swabs, urine for virus isolation and sera for serologic tests were taken concurrently from each infant and the mother when they consulted the doctor for the first time. Specimens were stored at —70 C and —20C, respectively.

Hemagglutinating antigen was prepared in monolayers of BHK21 cells infected with the M33 strain of rubella virus. The hemagglutination inhibition (HI) test was carried out in tubes (15 mm × 95 mm) by the original method of Stewart et al. (1967) with slight modifications. Sulfhydryl sensitivity of antibody activity was demonstrated immediately after treating the sera with an equal volume of 0.2 M 2-mercaptoethanol (2-ME) for 1 hour at 37 C and without dialysis (Sekiguichi et al., 1968). Tests on 2-ME treated and untreated sera were made in parallel. Serum was regarded as

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sensitive when two-fold or more reduction in the antibody titer was observed after this treatment. Sedimentation analysis of rubella antibodies in serum was performed by the method of Schluederberg (1965) and Vasikari et al. (1968). A 0.3 ml sample of kaolin-treated serum was layered on 4.5 ml of a linear gradient of 10–37% w/v sucrose and centrifuged at 35,000 r.p.m. for 15 hours in a Spinco L SW39 rotor. Twenty three fractions were collected dropwise by piercing the bottom of the tube. Attempts were made to isolate virus by the indirect interference method described by Parkman et al. (1964). Volume of 0.2 ml of throat swabs and urine were inoculated into six tubes (15 mm×95 mm) containing primary green monkey kidney (pGMK) cell cultures. The culture fluids from the six tubes were pooled and subpassaged on the 7th day. After the second incubation for seven days, the pGMK tube cultures were tested for rubella virus interference using about

TABLE 1. *Clinical findings on seven suspected cases of congenital rubella*

Case	Maternal History	Clinical Findings of Infants				
		Birth Weight	Heart	Eye	Ear	CNS & Others
M.F.	eruption in the 2nd month of gestation, delivery at the 40th week of gestation	2540 g	ventricular septal defect	uni-cataract, retinopathy, microphthalmia	difficulty in hearing	microcephaly, mental retardation, hepato-splenomegaly
Y.N.	eruption in the first month of gestation, eruptive infection prevailed in the neighborhood, delivery at the 37th week of gestation	1680 g	ventricular atrial septal defects, pulmonary artery stenosis	bi-cataract, retinopathy, microphthalmia		hepatomegaly
T.S.	eruption in the 3rd month of gestation, eruptive infection prevailed in the neighborhood, delivery at the 40th week of gestation	1300 g	pulmonary artery stenosis	uni-cataract, retinopathy, microphthalmia		mental retardation
T.I.	eruption in the 4–5th month of gestation, eruptive infection prevailed in the neighborhood, delivery at the 40th week of gestation	2957 g			deafness	cerebral paralysis, abnormal metaphysis of long bone
H.O.	subclinical, eruptive infection prevailed in the neighborhood, delivery at the 40th week of gestation	2300 g	ventricular septal defect	bi-cataract, microphthalmia	deafness	mental retardation
M.D.	eruption in the first month of gestation, eruptive infection prevailed in the neighborhood, delivery at the 40th week of gestation	2560 g	patent ductus arteriosus	bi-cataract		hepatomegaly, cerebral paralysis
K.I.	eruption in the 3rd month of gestation, delivery at the 41st week of gestation	2380 g	noise Levine II	uni-cataract	difficulty in hearing	

200 $TCID_{50}$ of ECHO virus type 11.

Tables 1 and 2 summarize the clinical and laboratory findings on the seven suspected cases of congenital rubella in Osaka.

In the early stage of pregnancy, six mothers experienced an eruptive infection and the other mother came in contact with rubelliform patients but developed no symptoms of the disease. The maternal anamneses coincided with periods of prevalence of eruptive infection in the neighborhood. In addition to these anamneses, the rubella HI antibody titers of the maternal sera examined were high, suggesting that the eruptive infection might be due to rubella virus. Clinical findings on the infants show congenital anomalies characteristic of the congenital rubella syndrome, such as a low weight at birth, ventricular and/or atrial septal defects, patent ductus arteriosus, pulmonary artery stenosis, cataract, retinopathy, microphthalmia, deafness, mental retardation or their various combinations. The rubella HI antibody titers of the sera from five of the infants were equal or greater than those collected at the same times from the mothers. Thus the infants were infected with rubella virus at an embryonic stage and produced their own rubella HI antibody consisting of IgM fractions before birth or in the neonatal period (Alford, 1965: Bellanti et al., 1965: Soothill et al., 1966), when they were usually protected from natural rubella infection by transplacental transfer of maternal antibody. If the infants had not themselves produced the antibody, the antibody levels of their sera would be lower, or at most the same as those of the maternal sera. We could not decide from serological results whether two cases aged 12 and 21 months suffered from congenital rubella, because at these ages maternal antibody must

TABLE 2. *Virologic and serologic findings on seven suspected cases of congenital rubella*

Case	Age of Infant Tested	Subject	Rubella HI Antibody Titer			Sedimentation Analysis		Rubella Virus Isolation from Infant
			Untreated	2-ME Treated (Whole Serum)		IgM Antibody Fraction	2-ME Treatment (IgM Fr.)	
M.F.	63 Days	Mother	1 : 256	1 : 256	R	(−)		Throat Swab (−)
		Infant	1 : 512	1 : 512	R	(+)	S	Urine (−)
Y.N.	41 Days	Mother	1 : 512	1 : 512	R	(−)		Throat Swab (−)
		Infant	1 : 512	1 : 512	R	(+)	S	Urine (−)
T.S.	21 Months	Mother	NT	NT		NT		NT
		Infant	1 : 64	1 : 64	R	(−)		
T.I.	7 Months	Mother	1 : 256	1 : 256	R	NT		Throat Swab (−)
		Infant	1 : ≥1024	1 : ≥1024	R	(−)		Urine (−)
H.O.	12 Months	Mother	1 : 512	1 : 512	R	NT		Throat Swab (−)
		Infant	1 : 256	1 : 256	R	(−)		Urine (−)
M.D.	34 Days	Mother	1 : 256	1 : 256	R	(−)		Throat Swab (−)
		Infant	1 : 256	1 : 128	S	(+)	S	Urine (−)
K.I.	38 Days	Mother	1 : 256	1 : 256	R	NT		Throat Swab (−)
		Infant	1 : 512	1 : 512	R	NT		Urine (−)

R : resistant, S : sensitive, (+) : detected, (−) : not detected, NT : not tested

have been disappeared and they might have experienced natural infection of rubella. Moreover, even in cases of congenital rubella, infants may have produced predominantly IgG rubella antibody and may have replaced the IgM antibody by IgG antibody. To determine whether congenital anomalies of patients were caused by rubella virus infection during an embryonic stage, it is necessary to isolate rubella virus from patients during early infancy and/or to demonstrate IgM rubella antibody, which is not readily transferred through the placenta, in the serum of patients during early infancy (Cooper et al., 1966). No virus was isolated from throat swabs or urine samples of infants. This failure might be because specimens were taken too late for virus isolation. On the other hand, very high levels of IgM rubella antibody were demonstrated in the sera from three infants (M.F., Y.N. and M.D.) by sucrose density gradient sedimentation analysis but not in the sera of their mothers. Moreover, the rubella HI antibody titer of these IgM fractions was reduced by 2-ME treatment (Fig. 1).

Therefore, these three infants were confirmed as cases of congenital rubella syndrome by retrospective serologic studies. The sulfhydryl reactivity of the whole sera, in which the IgM rubella antibodies were detected by sedimentation analysis, can be explained from the ratio of the amount of the 2-ME resistant IgG rubella antibody to that of 2-ME sensitive IgM rubella antibody, as shown in Fig. 1. Only two-fold reduction in the rubella HI antibody titer was observed even in 2-ME-treated serum from one infant (M.D.). Thus, as already pointed out by Alford (1965), this

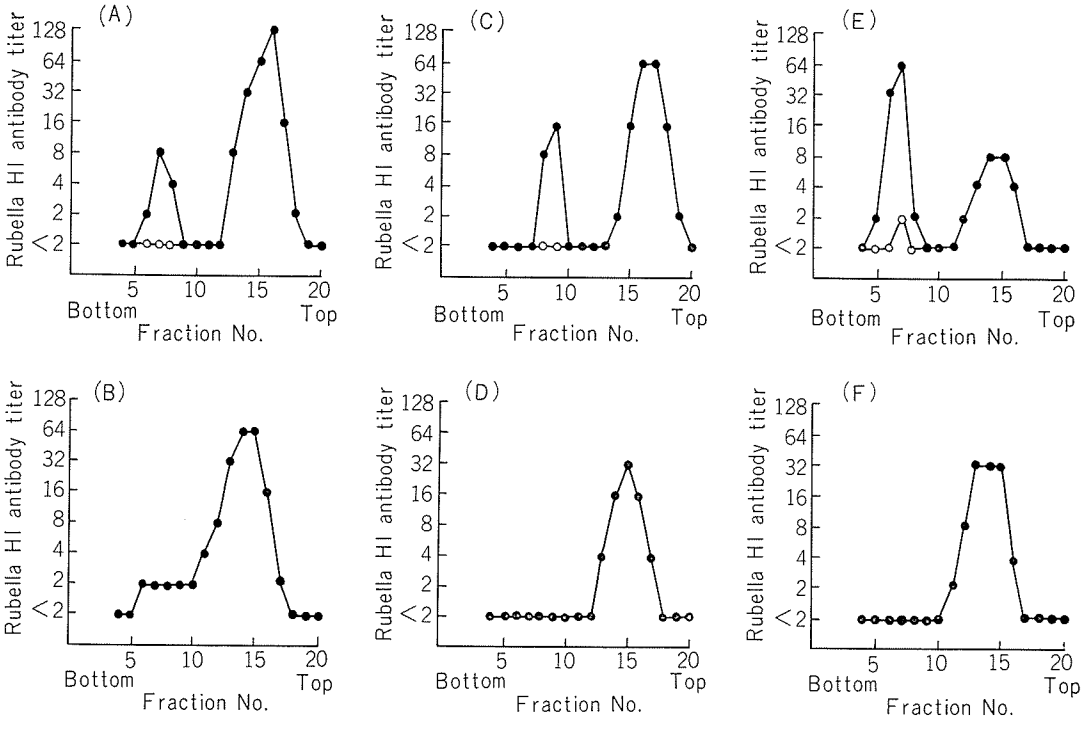

FIGURE 1. *Sedimentation analyses of rubella antibodies in sera collected from (A) M. F. Infant, (B) M. F. Mother, (C) Y. N. Infant, (D) Y. N. Mother, (E) M. D. Infant, and (F) M. D. Mother.*
● *Untreated,* ○ *2ME treated*

method is not very sensitive and is not recommended for demonstrating IgM antibody in whole serum of cases of congenital rubella.

Two explanations have been proposed for the lower incidence of congenital rubella in all parts of Japan except Okinawa. One is that the young adult population is in a more immune state against rubella (Suto et al., 1968: Sato et al., 1968), and the other is that rubella virus strains in Japan are much less teratogenic than American strains in experimental animals (Kono et al., 1969). The present results show that the rubella virus strain(s) in Osaka appears to have some teratogenicity and that cases of congenital rubella occur sporadically not as epidemics in Osaka, where most of the population has already acquired immunity to rubella before twenty years of age (Kimoto et al., unpublished data). To elucidate the factors affecting the difference in the incidence of congenital rubella in the United States and Osaka, epidemiological studies should be made on congenital rubella, especially in districts where the state of immunity against rubella is low. Moreover the teratogenicity of rubella virus isolated in Osaka should also be tested.

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